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LXIII. Band **Inhaltsverzeichnis** 4. Heft

The Ultrastructure of the Wreath Cells of *Drosophila melanogaster* Larvae

By

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With 7 Figures

(Received May 15, 1966)

Cells forming a ring which encircles the esophagus at its junction with the proventriculus were first observed in *Musca vomitoria* larvae by Weissman (1864) who named them "Guirlandenzellen" (wreath cells). The purpose of this paper is to describe the submicroscopic morphology of the larval wreath cells of *Drosophila melanogaster*, to compare them with cells of similar morphology residing in clusters near the proventriculus of the adult, and to speculate as to the function of these cells.

Materials and Methods

Drosophila melanogaster belonging to the inbred, wild type Oregon S strain were cultured upon David's medium (David 1962) at 21°–22° C in a normal cycle of daylight and darkness. Females that had spent either 46 ± 0.5 or 97 ± 0.5 hours in the larval stage provided the larval wreath cells studied. Individuals from the first group were in the terminal portion of the second instar; whereas those of the last group were in the terminal portion of the third (and last) larval instar. Within the next two hours larvae from the first group were due to molt, while those from the second group were due to molt and to form puparia. Similar "periproventricular" cells clustered near the proventriculus were obtained from mated 12 hour and 3 day old adult females.

All tissues were fixed in a solution consisting of 1% OsO_4 and 1% $K_2Cr_2O_7$ in 0.05 M sodium cacodylate buffer (pH 7.4) and left at 4° C for 2 hours. The infiltration was done at room temperature using acetone as the dehydrant and propylene oxide as the monomer solvent according to the Maraglass procedure of Spurlock, Kattine and Freeman (1963). During dehydration the tissue was stained for 10 minutes in 1%

$KMnO_4$ in absolute acetone in accordance with the procedure of Eakin and Westfall (1962). After an 18 hour polymerization period at 50° C the plastic block was trimmed, and sections giving grey interference patterns were cut with an LKB Ultrotome. These thin sections were stained with saturated, aqueous, uranyl acetate for 1.5 hours at room temperature and counterstained with lead hydroxide for 5 minutes according to the procedure of Feldman (1962). Electron micrographs were taken with a Hitachi HU11A electron microscope.

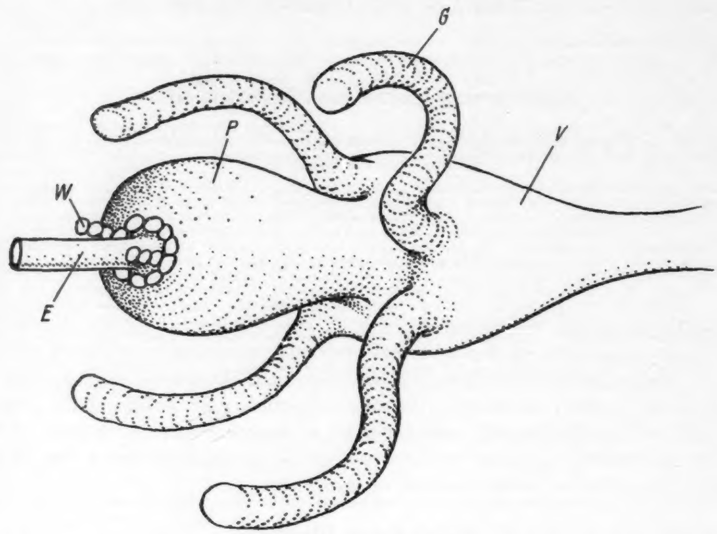


Fig. 1. A diagrammatic drawing of a portion of the larval digestive system of *Drosophila melanogaster*. *E* esophagus; *G* gastric caeca; *P* proventriculus; *V* ventriculus; *W* wreath cells. ×200.

Observations

In *Drosophila melanogaster* larvae clustered wreath cells form a small ring encircling the esophagus at its junction with the proventriculus (Fig. 1). A low power electron micrograph of a typical wreath cell is shown in Fig. 2. Its plasmalemma has invaginated extensively to form a system of small, thick walled tubules and vesicles. These may coalesce to produce the larger, membrane-enclosed vacuoles which lie deeper in the cytoplasm. Such vacuoles often contain dense deposits. Mitochondria and dense granules of similar dimensions lie still deeper in the cytoplasm. Wreath cells are mono- or bi-nucleate. Each nucleus contains a single nucleolus and is surrounded by a characteristic two-layered, annulate envelope. The rough surfaced endoplasmic reticulum is in the form of a perinuclear system of interconnected, branching moniliform tubules. The ribosome-studded, thin

walled vesicles and short tubules seen more peripherally may be budded off this perinuclear system. The cytoplasmic background contains suspen-

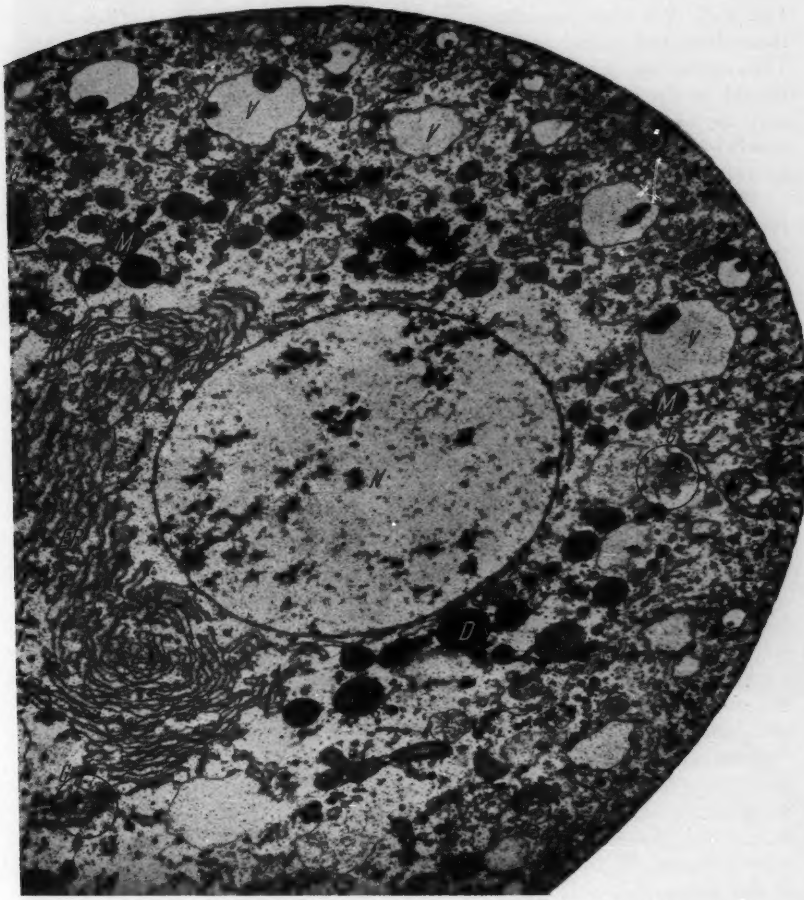


Fig. 2. An electron micrograph of a median section through a wreath cell from a third instar female larva. Endoplasmic reticulum (*ER*) lies against one side of the nucleus (*N*). The cortex of the cell contains numerous thick walled small tubules and vesicles. Large vacuoles (*V*), some of which contain dense deposits, lie deeper in the cytoplasm. Mitochondria (*M*) and dense granules (*D*) occur still deeper in the cell. The Golgi material (*G*) is encircled. ×9,140.

ded polyribosomes. The Golgi material consists of clusters of small thin walled, smooth surfaced vesicles. The large, ovoid wreath cells are held together by a network of filamentous, smooth muscle cells (Fig. 3).

The periproventricular cells of young adult females are very similar in appearance. They differ, however, in that a subpopulation of large vacuoles exists which were not observed in the larval sections. (Fig. 2, V vs. Fig. 4. V, V[1].) Such vacuoles appear denser in electron micrographs than those described earlier because their flocculent contents are more compact. They occur together with the vacuoles of lower density but are generally deeper in the cell. Their investing membranes are generally thinner and may be broken. They lack the peripheral, small dense deposits characteristically found in the vacuoles of the first type. Cytoplasmic granules occur as in larval wreath cells, but these are less dense. (Fig. 2, D vs. Fig. 4, D[1].)

Higher power electron micrographs of the cortical region suggest an intensive internalization of macromolecules adhering to the cell surface (Figs. 5 and 6). Cytoplasmic tubules about 50 mμ in diameter are formed by closing off cylindrical, dense rimmed depressions of the plasmalemma. The lips of these clefts are strengthened by desmosomal deposits (Fig. 7). Some tubules remain attached by one end to the plasmalemma while others seen 1–2 μ from the cell surface in sections are presumably floating free. Numerous pouch-shaped projections occur on the tubes and similar invaginations of the plasmalemma are seen. These pouches are dense rimmed. At magnifications in the neighborhood of 60,000 their walls show a honeycomb structure made up of subunits about 10 mμ in diameter.

Discussion

Early studies at the light microscopic level have demonstrated that certain cells, generally residing near the dorsal blood vessel of the insect and called pericardial cells or nephrocytes, absorb and concentrate injected substances (Hollande 1921, Lesperon 1937, Wigglesworth 1943, Palm 1952). Hollande was the first to postulate that pericardial cells absorb and store certain complex, toxic compounds present in suspension in the blood and subsequently transform these compounds to soluble, non-toxic molecules which are liberated into the blood for subsequent elimination by the Malpighian tubules. Studies of pericardial cells from hemimetabolous insects utilizing electron microscopy have demonstrated systems of cortical vesicles and tubules which suggest that the cells are undergoing extensive micropinocytosis (Kessel 1961, Bowers 1964). Similar findings were reported by Mills and King (1965) who studied the ultrastructure of the pericardial cells which occur in clusters adhering to the heart of 10 day old, adult, female *Drosophila melanogaster*. Mills and King also interpreted the cortical invaginations as a manifestation of an intensive and perhaps discriminatory internalization of the macromolecules seen adhering to the plasmalemma. Following Hollande's thesis they suggested that pericardial cells internalized potentially toxic molecules present in the blood and therefore that these cells were behaving like the reticuloendothelial cells of mammals. The present paper demonstrates that the wreath cells of both second and third instar female larvae have a morphology similar to that of adult periproventricular cells, and both types of cells closely resemble the abdominal pericardial cells of adults studied by Mills and King.

Fig. 3. An electron micrograph of a paramedial section through a wreath cell from a third instar larva showing points where smooth muscle cells (*S*) attach it to neighboring cells. ×13,150.

We suggest that the inclusive term pinocyte be used to refer to all the above pinocytotically active cells.

The pinocytes observed by Mills and King were from older flies which were reared on the medium of King and Wood (1955). They contained two classes of large, membrane-enclosed, cytoplasmic vacuoles which were referred to as B_1 and B_2 bodies. B_1 bodies have a similar morphology to the large vacuoles (Fig. 2, V) seen in wreath cells. B_2 bodies, which are characterized by crystalline deposits showing parallel longitudinal striations, have not been observed in the pinocytes examined during this study. Therefore B_2 bodies may arise later in adult life, or only when certain diets are employed, or the pinocytes surrounding the heart may differ physiological from those surrounding the proventriculus. Mills and King also observed particles (A bodies) which they thought resembled lysosomal storage granules. It was postulated that these lysosomes coalesced with the vacuoles and in so doing donated their complement of hydrolytic enzymes. In this study lysosome-like particles were seen in large numbers only in the pinocytes from 3 day old adult females.

The alveolate nature of the cytoplasmic coating of the surface invaginations of pinocytes was first described by Bowers (1964) for the aphid, *Myzus persicae*. Bowers suggested that plasmalemmal invaginations bearing cytoplasmic coatings made up of closely packed subunits are associated with the uptake of protein by the cell. The coated area may represent a specially differentiated cortical site adapted for the binding of extracellular material and for subsequent plasmalemmal invagination and vesiculation.

We assume that the vesicles and tubules move internally once they are budded off the surface. They coalesce with one another and so form larger vesicles within which the internalized macromolecules are stored and later digested. The vacuoles seen in Fig. 4 may represent early (V) and later stages (V^1) in this process. The vacuole may subsequently break down within the cytoplasm, or if a large central vacuole exists in the cell (Mills and King 1965, their Fig. 2), they may coalesce with it.

Summary

The wreath cells of *Drosophila melanogaster* larvae are described and compared with cells of similar morphology residing near the proventriculus of the adult. Both types of cells closely resemble the abdominal pericardial

Fig. 4. An electron micrograph of the cytoplasm of a periproventricular cell from a 12 hour old adult female. The endoplasmic reticulum (*ER*) consists of interconnected, branching tubules which lie near the nucleus (*N*). Polyribosomes are visible in areas where the surface of this membranous system lies parallel to the plane of the section. Polyribosomes also lie free in the cytoplasm along with mitochondria (*M*). The Golgi material (*G*) consists of a cluster of tiny vesicles. Two types of large membrane-enclosed vacuoles occur. Vesicles of the first type (*V*) have a relatively low density; those of the second type (V^1) lie deeper in the cytoplasm, are denser, and their enclosing membranes are often ruptured. Deposits of medium density (D^1) are commonly seen. ×20,000.

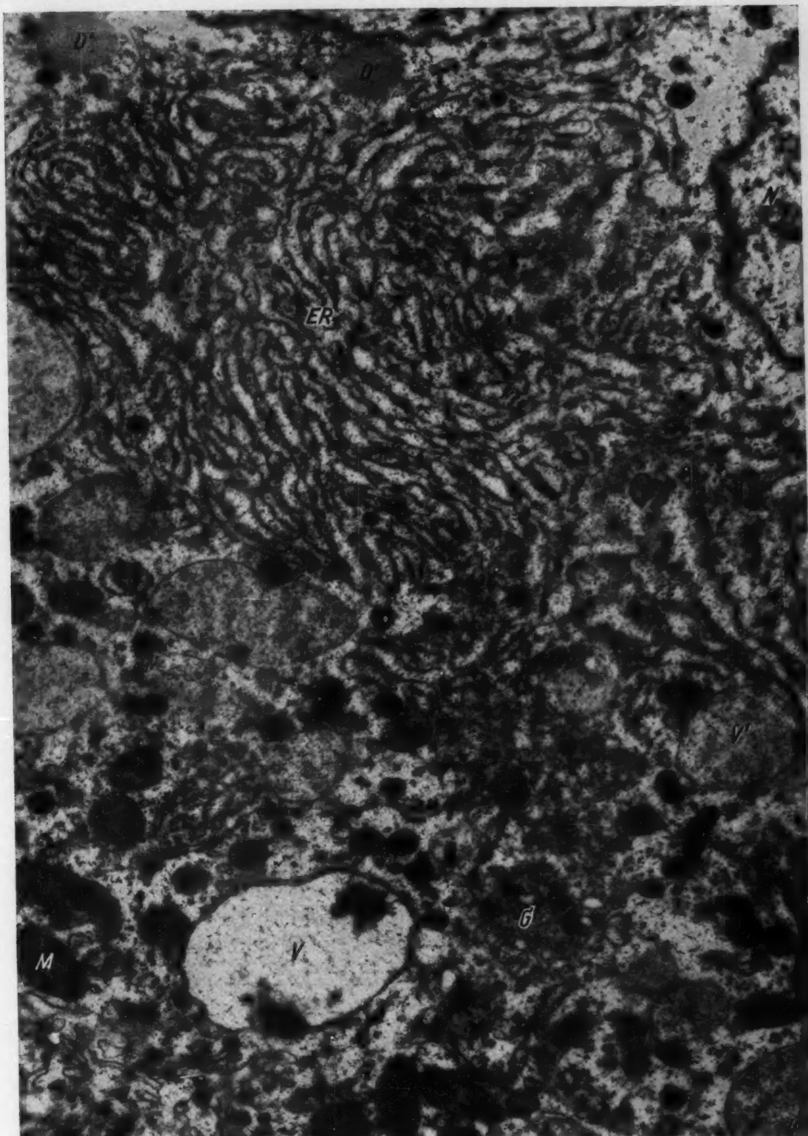
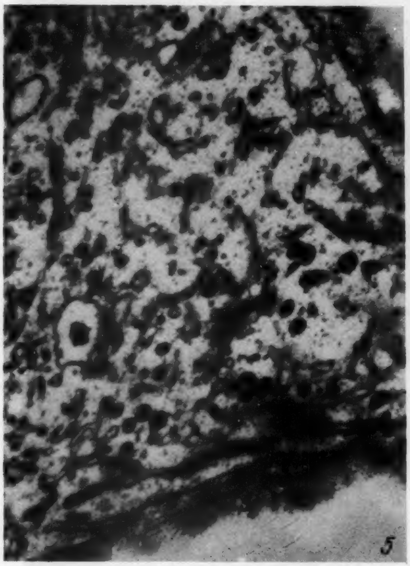


Fig. 4.

cells of adults. All possess systems of cortical vesicles and tubules which suggest that the cells are undergoing extensive micropinocytosis. Such cells are postulated to behave like the reticuloendothelial cells of mammals in that they internalize by micropinocytosis potentially toxic macromolecules



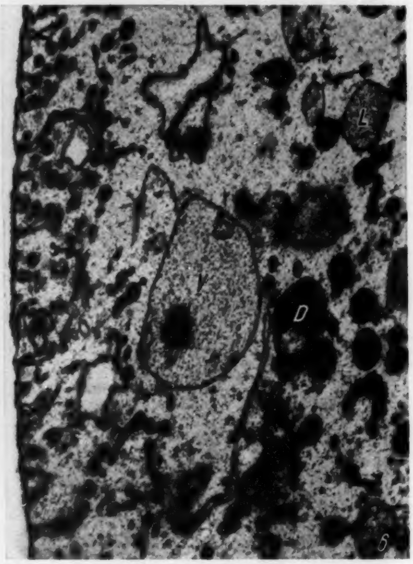


Fig. 5. An electron micrograph of a section cut tangential to the surface of a wreath cell from a second instar larva. A flocculent precipitate adheres to the cell surface (*S*). Some invaginations of the plasmalemma take the form of undulating, dense rimmed clefts. Cytoplasmic tubules are plentiful. Many have blister-like terminations, and pouch-shaped projections at various intermediate points. Abscission of these pouches produces dense rimmed vesicles which are also abundant. ×20,000.

Fig. 6. An electron micrograph of the edge of a periproventricular cell from a 3 day old adult female. In this figure the clefts are generally seen in cross section; whereas in Fig. 5 they appear in surface view. Many incidences of the abscission of dense rimmed vesicles can be seen. Vacuoles (*V*), dense granules (*D*), and lysosomal storage granules (*L*) occur in the deeper cytoplasm. ×20,000.

and concentrate and store them in membrane-enclosed vacuoles. These deposits may later be transformed into non-toxic, soluble molecules and liberated. It is suggested that the term p i n o c y t e be used to refer to the above pinocytotically active class of cells.

Acknowledgments

The investigation was supported by the U. S. Public Health Service (grant GM 9694), the National Science Foundation (grant GB 2429), and the American Cancer Society (grant E 408).

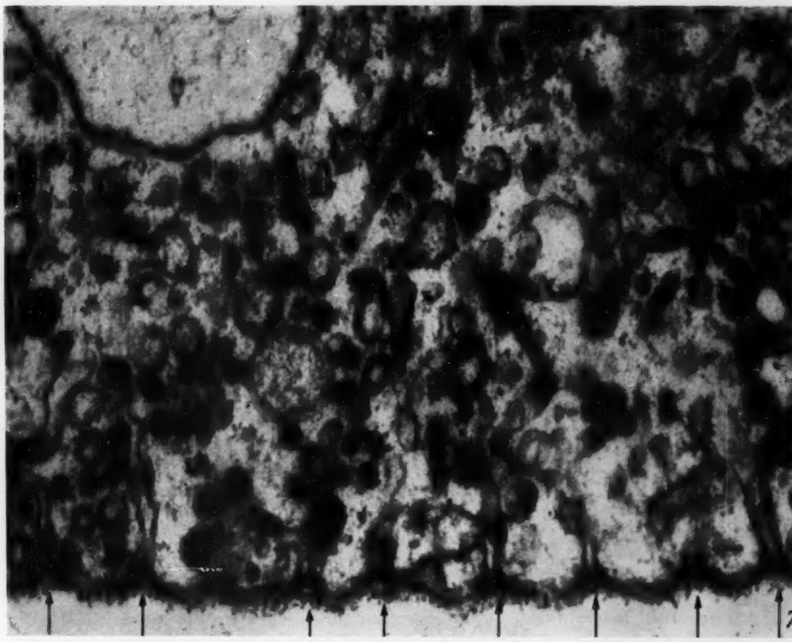


Fig. 7. An electron micrograph of the edge of a wreath cell from a third instar larva. Note the flocculent precipitate adhering to the cell surface. Arrows point to desmosome-like modifications of the plasmalemma at the points of invagination. In the cytoplasm clusters of ribosomes are observed as well as the profiles of sectioned tubules and vesicles. ×60,000.

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The Fine Structure of Mucilage Secreting Cells of *Hibiscus esculentus* Pods

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With 12 Figures

(Received June 20, 1966)

Introduction

Plant mucilages are complex polysaccharide polymers of high molecular weight. They may be derived from roots, leaves, seeds and flowers of plants. They are normal products of metabolism and may serve (like starch) as reserve food sources within the plant. They may also serve as regions for water retention. All natural mucilages are either acidic or neutral polysaccharides [6].

One of many mucilage producing plants, the okra was chosen for study because it is easily attainable and is amenable to the preparative procedures necessary for ultrastructural analysis. The mucilage of the okra pod is viscous and similar in appearance to the slime of the maize root cap. It is present in large quantities and has been analyzed chemically. This study was undertaken, therefore, in an attempt to relate an ultrastructural pattern to slime secretion.

Materials and Methods

Okra (*Hibiscus esculentus*) pods in various developmental stages were excised from the plant and hand sectioned into thin transverse slices near the mid region of the pod. The thin slices were immediately placed into cold, cacodylate buffered, 3 percent acrolein—3 percent glutaraldehyde mixture. After approximately 1 hour in the acrolein-glutaraldehyde mixture, the tissues were rinsed in 4 changes of cold buffer for 10 minutes each followed by a 1 hour post fixation in cold, buffered, 1 percent OsO_4. The tissues were then rinsed in distilled water and dehydrated in an alcohol series followed by 3 changes of 100 percent acetone. They were then embedded in No. 2 Epon-Araldite mixture as reported previously [2]. Sec-

tions were cut with diamond knives and scanned with a Philips 200 microscope.

Light microscopy utilized ½–2 μ sections cut from the acrolein-glutaraldehyde, OsO_4-fixed, epoxy-embedded tissue segments. The sections were mounted on glass slides and stained for polysaccharide by the Periodic acid-Schiff reaction [1].

Results and Discussion

Light Microscopy

The Periodic acid-Schiff reaction was effective in staining selective areas within the okra seed capsule (Fig. 1). Of particular interest were numbers of very large cells located within the outer portion of the pericarp (Fig. 1). These cells were heavily stained, including the small bit of stranded cytoplasm within them. In sections cut transverse to the long axis of the okra pod, these large cells appeared singly or in groups of 2–8. The individual cells, or groups of cells, were not interconnected but appeared to be totally surrounded by cells of another type. Whether they remain isolated throughout development is not known. No other cells of the okra pod were conspicuously stained.

Electron Microscopy

There is not sufficient space to adequately illustrate all cells of the okra pod and therefore some selection has been made. Emphasis is upon cells of the pericarp which might be engaged in secretion.

The majority of cells, at the developmental stage studied, are highly vacuolate and have only a thin peripheral rim of protoplast containing nuclei, mitochondria, chloroplasts (usually with only a small number of grana), dictyosomes and endoplasmic reticulum (Figs. 2–5, 10). Occasionally, some of these cells are seen in division. The ultrastructure of cells of the receptacle tissues (i.e. tissues of the pod axis) and of the ovule are not included in this report.

Tracheary Elements

The tracheary elements of the okra pod are not unusual in form or content. However, they are involved in secretion of cell wall components and their development can be easily followed. The discussion of them will be confined to the relationship between the Golgi apparatus and the cell wall.

Several features relative to wall formation are apparent. First, a structureless and translucent substance is deposited outside of the plasma membrane at the site of spiral thickening (Figs. 6, 7). Subsequently, a dense, fibrous, material begins to appear within the central part of the translucent substance (Figs. 6, 7). This dense, fibrous, material (presumably cellulose) is apparent first in the region of the primary wall (Fig. 6) and then develops inward toward the protoplast of the cell. The earliest visible

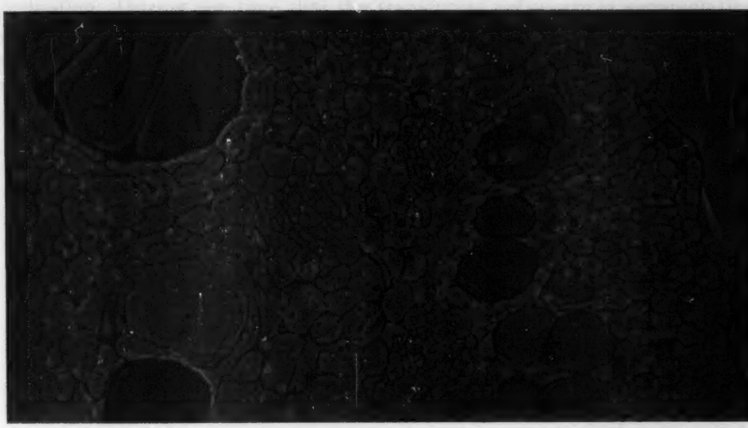

Fig. 1. A transverse section through the outer portion of a young okra pod stained for polysaccharide by the Periodic acid-Schiff reaction. The large secretory cells of the pericarp are conspicuous because of their size and staining reaction. ×160.

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fibers seem to form directly upon, or parallel to, those of the primary wall. The impression is that the fibers of the primary wall (or some related portion of them) act as orientation templates during initial polymerization of secondary wall fibers.

As the wall matures, the ratio of fibrous material to translucent amorphous substance increases until finally, at wall maturity, only the dense fibrous matrix is present (Fig. 8, 9).

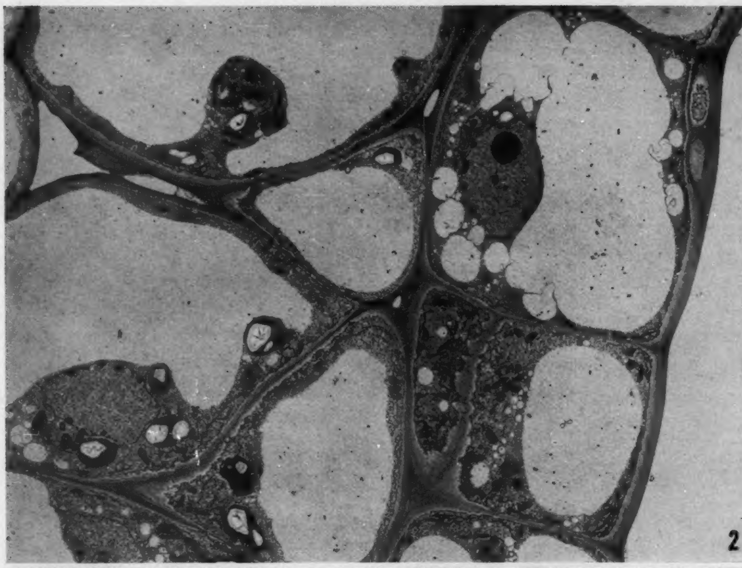
A sequence of dictyosome changes can be correlated with secondary wall formation. During the time that the translucent substance is accumulating at the site of spiral thickening, the dictyosomes are highly vesicular and obviously active in the segregation of secretory material (Fig. 6, 7). This secretory material is translucent and amorphous and is similar in appearance to that of the initial secondary wall thickening. As the secondary wall matures, the dictyosomes become less vesicular until, at wall maturity, they are no longer engaged in secretion (Figs. 8, 9). This sequence of events supports the observation of Wooding and Northcote that the Golgi apparatus of the tracheary cells supplies a quantity of wall precursor material which accumulates at the site of secondary thickening [7].

Large Secretory Cells of the Pericarp

Very large secretory cells are found throughout the outer portion of the pericarp (Fig. 1, 10). The dictyosomes of these cells are large with greatly hypertrophied cisternae (Figs. 11, 12). During early stages of development, the product of the dictyosomes accumulates between the cell wall and the protoplast (Fig. 10–12). This product corresponds to the PAS positive material of light microscopy (Fig. 1) and is derived from the Golgi apparatus. Eventually these pockets of secreted product dissappear, Golgi apparatus secretory activity ceases, and the cells become vacuolate. Ultimately the cytoplasm also degenerates. This pattern of development is similar to that of maize root cap mantle cells [3, 4].

The primary purpose of this study was to localize the source of okra mucilage. To the unaided eye, the mucilage appears to accumulate within the locule. During excision of tissue segments much of this accumulated mucilage is lost. Following the Periodic acid-Schiff reaction, only the large secretory cells of the pericarp are heavily stained. Thus, these large secretory cells appear to be the most likely source of okra pod mucilage. How the accumulated product is dissipated from the secretory cells during late stages of development is not known, though it is tentatively assumed that it moves to the locules through intercellular space.

Okra mucilage is a polysaccharide acid consisting largely of D-galactose (80 percent), L-rhamnose (10 percent), and D-galacturonic acid (6 percent) ([6] and Jones and Morré, unpublished). This (i.e., the polysaccharide nature) would be in keeping with other observations of plant cell secretions [5]. Conversely, if the principal part of the okra pod mucilage is derived from the large secretory cells, then the above analysis is primarily an analysis of a Golgi apparatus secretion product.



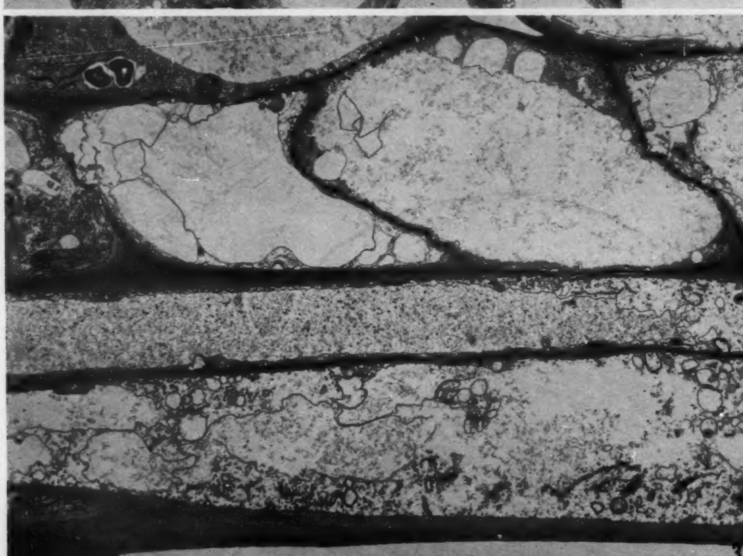
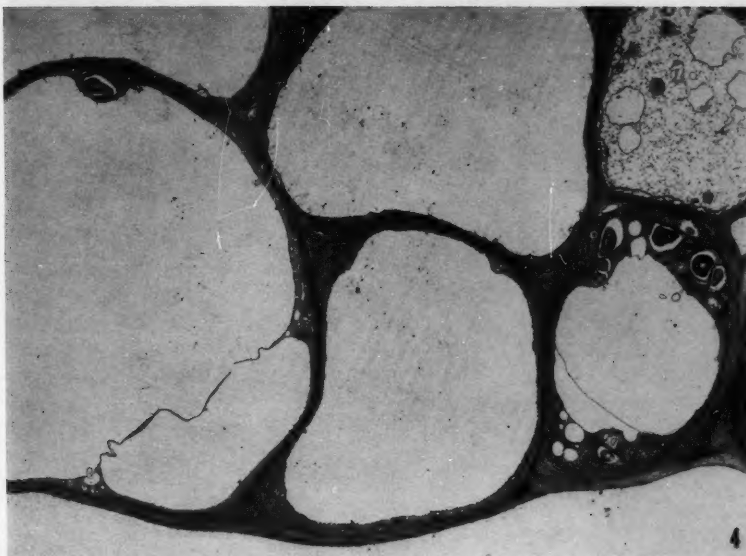


Fig. 2. A transverse section through cells of the pericarp showing their typical, highly vacuolate, nature. The epidermis is to the right. No conspicuous dictyosome activity was found in any of these cells. ×3,100.

Fig. 3. A transverse section through cells adjacent to the locule (a portion of locule is seen at the bottom of the micrograph). Some disruption of cytoplasmic components was apparent in most of the cells of this region. In some instances this was probably due to inadequate fixation. However, the consistent appearance of the cells adjacent to the locule suggests that they may have been degenerating. ×6,000.



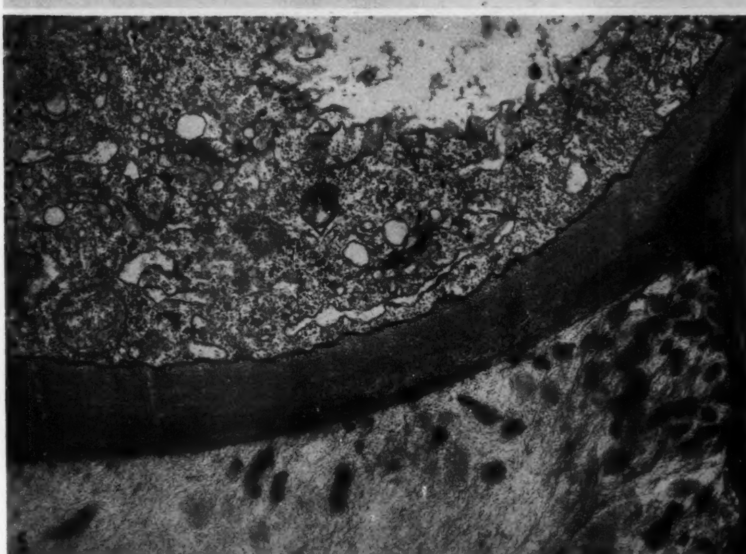


Fig. 4. Portions of cells adjacent to the cavity formed by the carpel walls (Note: Figs. 3 and 4 represent opposite sides of a carpel wall. Cells of Fig. 3 line the locule. Cells of Fig. 4 line the thin cavity formed by 2 carpel walls). ×4,300.

Fig. 5. A portion of a cell adjacent to the locule showing several dictyosomes with attached vesicles. Only a few such cells were encountered so it is not known whether, or to what extent, they may contribute to mucilage formation. The dense, sometimes fibrous, material in the locule is presumed to be mucilage. ×28,000.

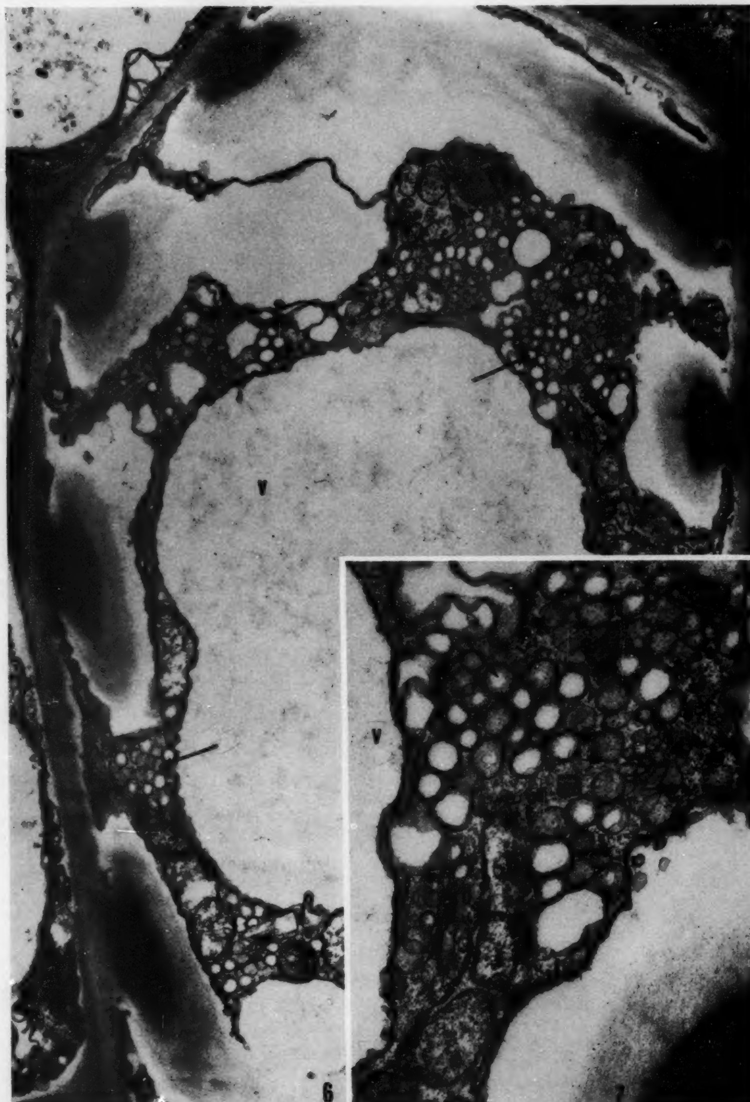
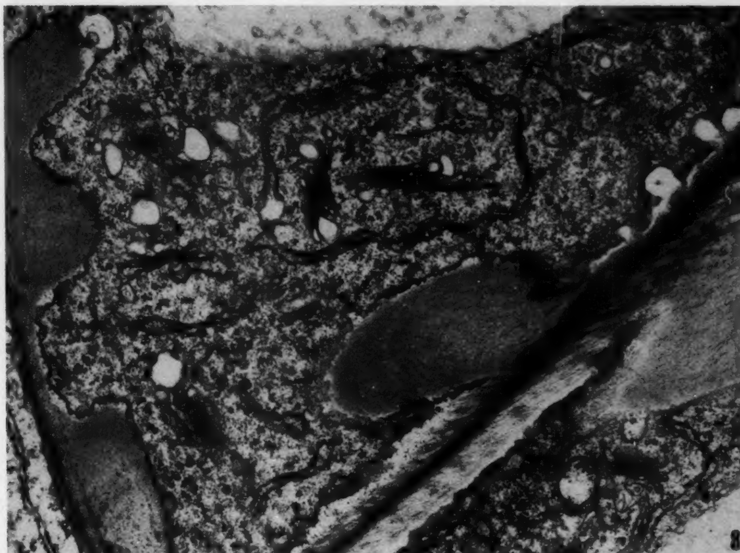


Fig. 6. A portion of a cell from the xylem showing regions of secondary wall thickening. The translucent substance is apparently derived from the Golgi apparatus and is, at least in part, the precursor material of the secondary wall. The polymerization of secondary wall fibers is first apparent in the central portion of the translucent substance and adjacent to the primary wall. The fibers of the secondary wall are oriented parallel to those of the primary wall. The arrows point to dictyosome-derived vesicles. *v* = vacuole, *d* = dictyosome. ×18,000.

Fig. 7. A portion of the same cell as Fig. 6 enlarged to show the dictyosomes (*d*) and dictyosome-derived vesicles (arrow). A portion of the secondary wall is seen at the bottom right of the micrograph. *v* = vacuole. ×43,000.



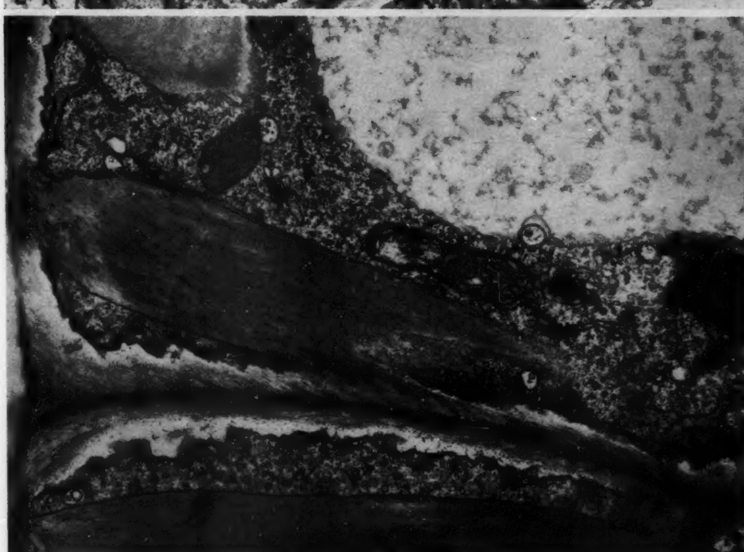
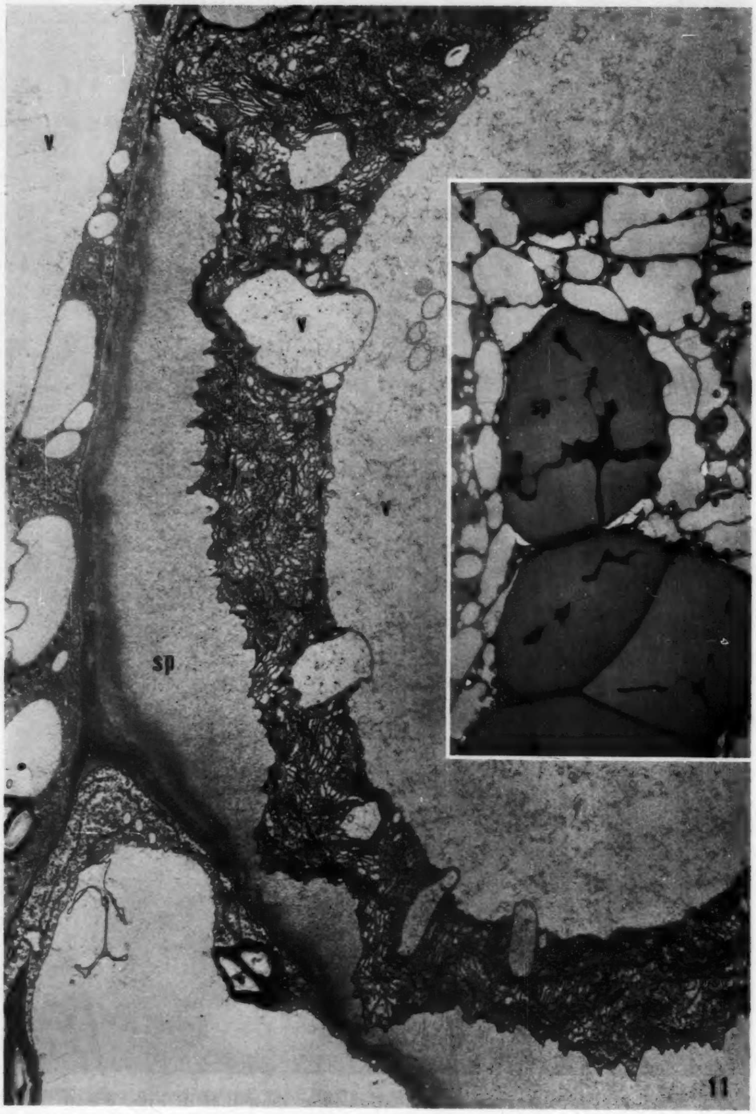


Fig. 8. A stage of secondary wall formation later than that of Figs. 6 and 7. The dictyosomes appear less active than previously and the polymerization of the fibrous wall material is nearly complete. ×30,000.

Fig. 9. A developmental stage later than that of Fig. 8 showing the last formative stage of spiral thickening. Dictyosomes are inconspicuous and few secretion vesicles are associated with them. ×26,000.



Figs. 10 and 11.

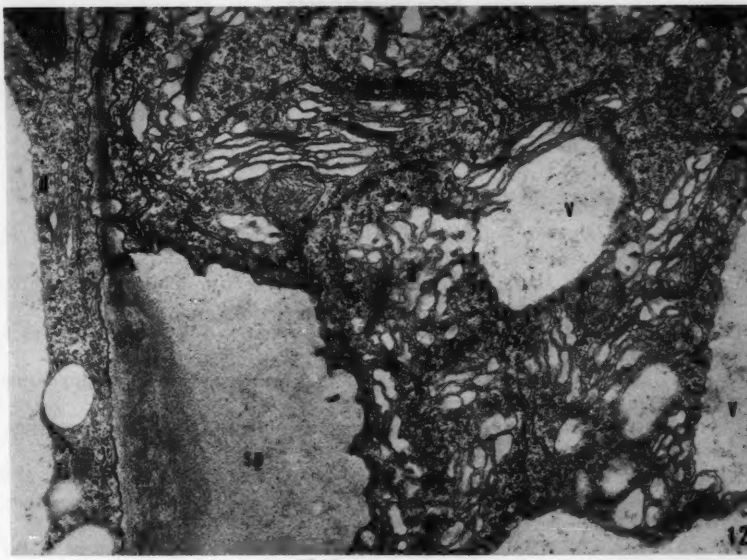


Fig. 12. A portion of the same cell as shown in Fig. 11 enlarged to show details of the dictyosomes (*d*), secretion product (*sp*) and vacuoles (*v*). A portion of a non-secretory cell is shown on the left. ×24,000.

Summary

Large secretory cells are found in the pericarp of okra capsules. In early developmental stages these cells are conspicuous because of the great quantity of Golgi apparatus-derived, PAS positive substance stored between the protoplast and the cell wall. It is suggested that these cells may play a major role in the formation of okra mucilage.

Fig. 10. A survey picture showing several of the large secretory cells of the pericarp. The dictyosome-derived secretion product (*sp*) occupies most of the cell volume and compresses the cytoplasm into thin, dense-staining bands. All other cells of the pericarp are highly vacuolate and none of them appeared to be as conspicuously active in mucilage secretion. ×250 (approximately).

Fig. 11. A portion of a large secretory cell at a developmental stage later than that shown in Fig. 10. The cells gradually become vacuolate and the secretion product (*sp*) moves into or through the cell wall. At a developmental stage later than that depicted here, the secretory cells contain a single large central vacuole, the dictyosome cisternae are no longer hypertrophied, and no secretion product is visible within or adjacent to the cell. This developmental pattern is similar to that of the maize root cap mantle cells [4]. *v* = vacuole. ×8,200.

Acknowledgements

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Charles F. Kettering Contribution No. 251.

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A Study of Salt Secreting Cells in the Brine Shrimp *(Artemia salina)*

By

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With 15 Figures and 1 Drawing

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Introduction

For the study of the physiological mechanisms of osmoregulation, it is logical to choose forms capable of surviving under extremes of environment. Thus, it is desirable, for example, to investigate the mechanism(s) that permit some aquatic animals to survive tonicities approaching that of distilled water and others to survive in concentrated brine. An example of the former case is the mosquito larva, which can live in rain water of varying purity. Whereas, an example of the latter case is the brine shrimp, which can survive in brine saturated to the point that salt crystalizes.

A study has already been made of *Culex* mosquito larvae reared from egg raft to fourth instar in water of 0.009 parts per thousand (‰) total salinity (Copeland 1964). The following constitutes a study of the fine structure of the metepipodite segments of the brine shrimp, *Artemia salina*, raised from eggs to adult forms in triple strength sea water (105‰ total salinity). Preliminary reports have been made (Copeland 1965 and 1966).

Materials and Methods

Artemia were raised from eggs distributed by the San Francisco Aquarium Society. Eggs represented as coming from Great Salt Lake, Utah, also were tested but the general survival was not as good and the adult size was smaller. Initial hatching was achieved in a finger bowl placed beneath a 60 watt goose neck light to raise the temperature slightly. Desired numbers of nauplii were transferred to the rearing chambers, which were

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glass battery jars holding one to two gallons of water, placed in the direct sunlight of a south window. No aeration was used.

Food in the form of Fleischmann's active dry yeast was sprinkled on the surface of the water. The jar was covered with a sheet of glass. The best growth was obtained in rearing chambers in which algal growth could be induced. However, in concentrations approaching 100‰ salinity it is increasingly difficult to induce floral or faunal growth and at 140‰ salinity the yeast remains as the only apparent food. *Artemia* raised at the latter concentration take about twice as long to reach maturity and the adults are slightly smaller than those raised at sea water strength (35‰ salinity).

Artemia were raised also in brackish water of 7‰ salinity. This proved to be a marginal condition for growth and not all cultures survived. Therefore, with the above observations in mind, the extremes of 10 and 105‰ salinities were chosen as concentrations in which large, active adults could be obtained in the laboratory. Mature adults from both concentrations were fixed for study of fine structure.

Artemia are extremely difficult to fix for fine structure studies. In our first attempts, several whole animals were dropped directly into chilled 5% glutaraldehyde and placed in a refrigerator over night. The next morning were still active although slowed by the cold. The procedure that we have developed is therefore described in some detail.

As a basic ingredient for fixation, glutaraldehyde (Sabatini et al. 1963) is preferred. For the buffer system, collidine (Bennett and Luft 1959), cacodylate (Tice and Barnett 1962) and phosphate (Millonig 1961) were used with almost equal success. The phosphate buffer seemed to give more consistent results. The osmotic pressure of the fixative is critical and sucrose (Caulfield 1957) was added to increase the pressure of all fluids used before the process of dehydration by ethanol. Osmotic pressures were determined by use of a Fiske Osmometer (freezing point depression) and recorded as milliosmoles. Strengths from 350 to 1400 milliosmoles were tested and the 700 to 900 range proved best for the animals raised in 105‰ salinity.

The following sample protocol also includes the use of acrolein (Luft 1959) combined with glutaraldehyde following a suggestion of Dr. Ned Feder. It was his observation that acrolein and glutaraldehyde do not start precipitating reaction until some 30 to 45 minutes after being mixed. Fixative solutions and alcohol dehydration to 95% were maintained at 4° C. All final solutions in steps 1 to 4 inclusive were adjusted to about 750 milliosmoles by the addition of sucrose. This required as much as 18 to 20% sucrose to be added to the buffer in step 3.

1. An animal is placed in a drop of fixative on chilled dental bite wax. Both head and posterior end are quickly removed with a small piece of razor blade held in a rat nose heamostat. Fixation is for 30 minutes in the following: 3% glutaraldehyde (pretreated with excess of barium carbonate) in Millonig's phosphate buffer adjusted to pH 7.3. Acrolein is added to the 2% level just before use.

2. The slightly hardened thorax is now cut sagitally and the halves oriented with the metepipodites against the bite wax. A downward cut

will remove most of the telopodites and cut the tips off at least some of the metepipodites. At this stage the cut surfaces usually remain open, aiding penetration of the fixative to the internal tissue. Fixation is continued for 2–3 hours in 3% glutaraldehyde in phosphate buffer.

3. The tissue is rinsed in repeated changes and left over night in Millonig's phosphate buffer.

4. Postosmication is done for one hour in 2% osmic in Millonig's buffer.

5. Dehydration is started at 30% ethanol and carried to 100% ethanol in about ½ hour. Dehydration in 100% can proceed for several hours, during

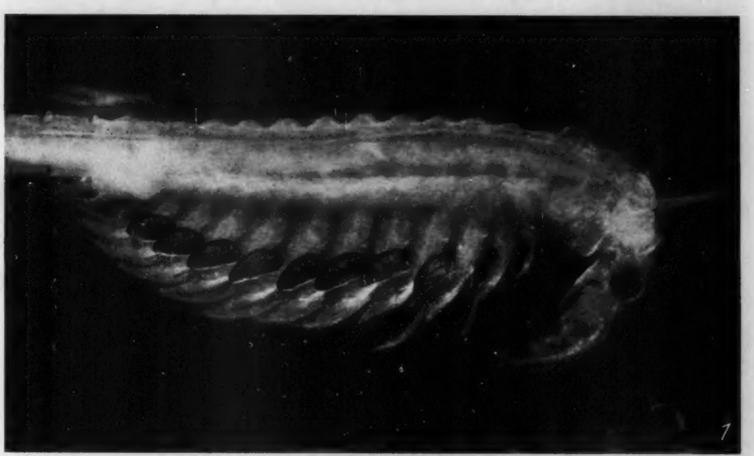
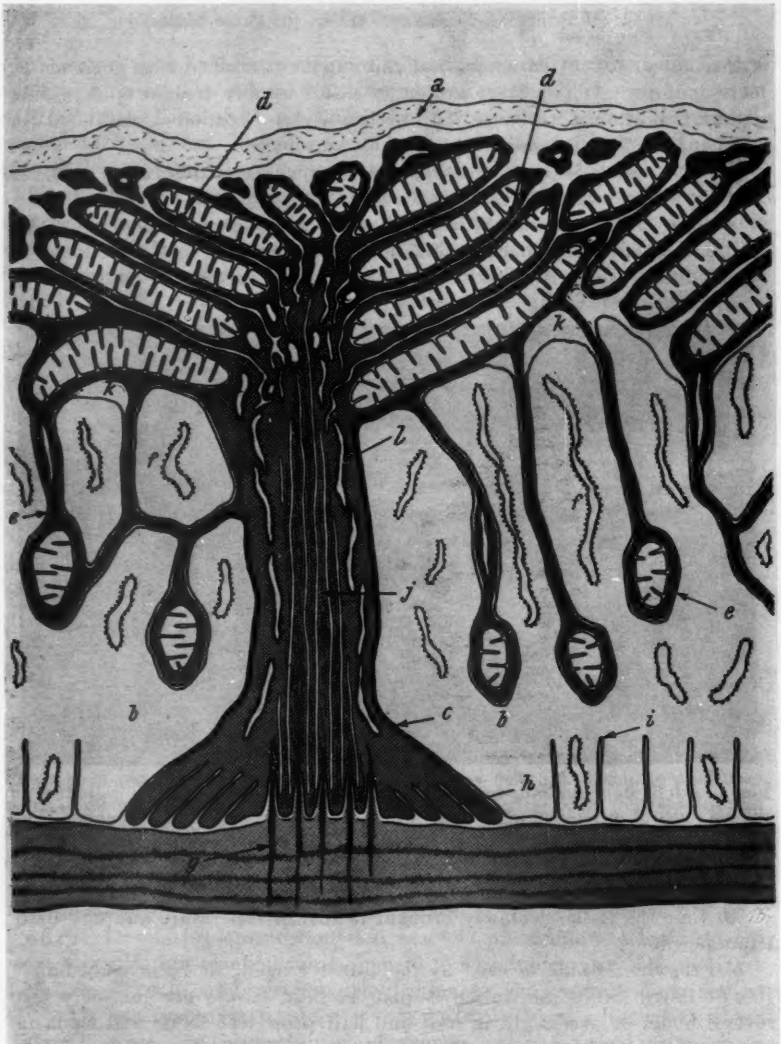


Fig. 1. Adult male *Artemia* treated with $AgNO_3$. The dark reaction is limited to the ten pairs of metepipodite segments which are flattened, leaf-like structures. They can also be identified as the only segments of the phyllopodia that do not have setae. ×20.

which time the tissue is slowly brought to room temperature and any final trimming done.

6. Propylene oxide is used as the transfer agent to Epon embedment (Luft 1961). Since infiltration is poor at best, tissues are routinely left several hours or over night in half and half propylene oxide and Epon in an open vial on top af a warm oven (about 35° C). The tissue is then transferred from the thickened plastic through several changes of catalyzed plastic and finally placed in gelatin capsules.

Even with attention to detail, fixation quality was not predictable and seemed to depend on the metepipodites being cut in such a fashion that fixatives could penetrate. If cut too early in the process of exposure to fixative, the cut ends tend to stay sealed. Also, infiltration of embedding media was frequently incomplete and some of the best sections showed occasional holes that opened further under the electron beam (Figs. 8 and 10). Use of a vacuum oven to facilitate penetration proved of no benefit.



Drawing 1. This schematic drawing depicts the interdigitation of the light and dark cell cytoplasms and the general arrangement of the mitochondrial pump systems in the dark cell. The cuticle is below. Haemolymph spaces and the granular basement membrane (*a*) are at the top. The dark cell is centrally located. Light cell cytoplasm is indicated at (*b*), dense membrane at (*c*), mitochondrial pumps at (*d*), dark cell projection with mitochondria at (*e*), light cell interdigitation with rough ER at (*f*), anchor fibers at (*g*), basal indentations, for dark cell at (*h*), and for light cell at (*i*). Microtubules and microfibrils are marked by (*j*) and intercellular spaces at (*k*). Rough endoplasmic reticulum of the dark cell is at (*l*). The figures should be consulted for details, using this diagram for orientation purposes.

A number of investigators have used $AgNO_3$ to demonstrate selectively various body areas of aquatic invertebrates. The earlier efforts were linked to the interpretation that blackening with silver indicated a vital reducing activity concerned with respiratory processes (Gicklhorn and Keller 1925, Dejdar 1930). More recent investigators consider the blackening to be indicative of salt (or chloride) movement through that area of the cuticle (Koch 1938 and Wigglesworth 1938). We used the $AgNO_3$ method outlined by Croghan (1958c) as a means of identifying and

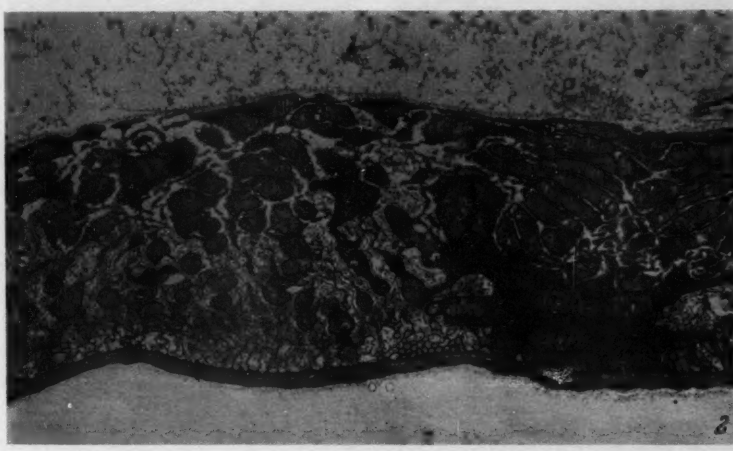


Fig. 2. General morphology in cross section of the segment. The cuticular surface is at the bottom and the haemolymph or lumenal surface of the metepipodite tissue is at the top, bound by a granular basement membrane. To the right is a dark cell body with mitochondrial pumps. The rest of the photograph is occupied by an interdigitating complex of light and dark cell cytoplasm with almost all the mitochondria located in the thin projections of the dark cell.

orienting the metepipodite segments until we became familiar with the animal (Fig. 1). Osmium tetroxide will blacken the segments but not as selectively.

Culture solutions with osmotic pressures above that of normal sea water were prepared by adding uniodized salt to sea water. The salt was usually obtained as "rock salt" or "ice cream freezer" salt. Salinity determinations were made with a direct reading resistance meter (Industrial Instruments, Solu Bridge RB 3-349).

Observations

The metepipodite segment of *Artemia* is a flattened pad or leaf-like structure (Fig. 1) with a finely dimpled surface. The internum of the cuticle is lined with a single layer of cells which can be classified into two types on the basis of the relative opacity to electrons. Thus, we will describe cells of low electron density and cells of high electron density,

which occur in about equal numbers and are arranged in an alternate, interdigitating manner. These will be referred to as light and dark cells respectively. Drawing 1 depicts the general pattern of the interdigitation and the location of the mitochondrial pumps to be described below. Drawing 1 will not be referred to again and should be used by the reader as needed to orient the detail of the text figures.

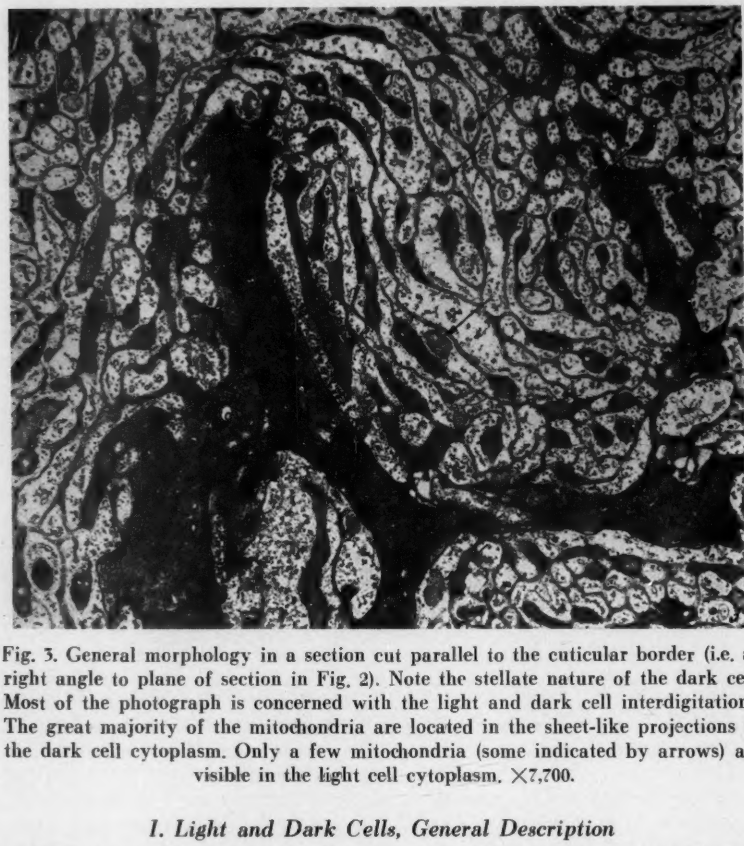


Fig. 3. General morphology in a section cut parallel to the cuticular border (i.e. at right angle to plane of section in Fig. 2). Note the stellate nature of the dark cell. Most of the photograph is concerned with the light and dark cell interdigitations. The great majority of the mitochondria are located in the sheet-like projections of the dark cell cytoplasm. Only a few mitochondria (some indicated by arrows) are visible in the light cell cytoplasm. ×7,700.

1. Light and Dark Cells, General Description

The two types of cells in addition to the distinct difference in density show marked differences in morphology that are not obvious at first glance because the extensive interdigitations make it difficult to delineate the boundaries of individual cells. In general the dark cells are columnar in disposition (Fig. 2). Frontal sections (i.e. parallel to the cuticle) show the dark cells in the form of stellate flanges that surround the light cells (Figs. 3 and 12). The cytoplasm of the dark cell is separated from that of the light cell by two apposed plasma membranes. In the region between the two cell

types, extending from the cuticular area to the level of the first cellular interdigitations, the space between the two plasma membranes is filled with a dense material (Fig. 5).

Fig. 4.

Fig. 5.

Fig. 4. Light and dark cell interdigitation; dark cell body to left. Sheet-like projections of the dark cytoplasm interdigitate with light cytoplasmic elements (see Fig. 5 for the other dimension of the sheets). Some projections arise from the main body of the dark cell (*a*), others arise as continuations of the mitochondrial pump system (*b*). One of the mitochondria (*c*) in the pump system is twisted out of the plane of the others, giving some indication of the pancake-like dimensions of the pump mitochondria. A dark membrane separates the main bodies of the light and dark cells. It connects to a basal indentation by a terminal bar or desmosome (arrow). ×6,300.

Fig. 5. Dense membrane separating light and dark cells. The dense membrane arises from a simple desmosome (zonula occludens) at the end of one of the basal indentations (*a*) and develops a periodicity (septate desmosome) at the point of transition into the two plasma membranes bounding the first cytoplasmic extension of the dark cell cytoplasm into light cell (*b*). See Fig. 4 for orientation. Also note that the membrane space of the basal folds is filled with granular amorphous material (secretory precursor of the cuticle?). ×44,100.

Thin cytoplasmic projections of the dark cell extend between evaginations of the light cell and such projections are easily identified by their density, morphology and concentration of mitochondria. The projections are really sheet-like and at intervals they bifurcate to form a complex that surrounds the thicker, finger-like complementary elements of the light cell (Figs. 3 and 4). As already noted, the dark cell projections include many mitochondria. The light cell extensions on the other hand have few or none.

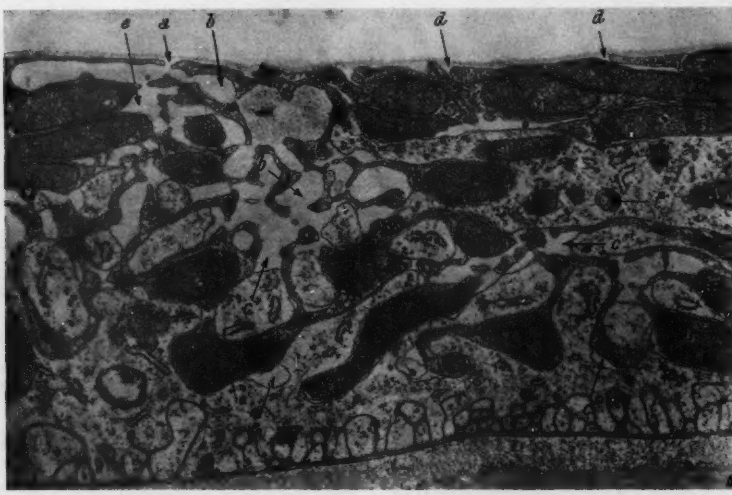


Fig. 6. Detail of the sinusoidal and intercellular spaces. The sinusoidal system communicates to the haemolymph by pores (*a*). The sinusoidal system (*b*) is limited to the dark cell but communicates freely with intercellular spaces (*c*). The lamellar spaces of the mitochondrial pumps may connect almost directly to the haemolymph surface (*d*) or to the sinusoidal system (*e*). Note one of the few mitochondria that can be identified as being in the light cytoplasm (*f*). ×16,800.

Extremely close associations of extensions of the membrane of the dark cells with flattened mitochondria are common and will be called "mitochondrial pumps" (Figs. 2 and 8). Sinusoidal spaces, which are extensions of the extracellular space, ramify through the dark cell and penetrate its lateral surfaces to connect with the intercellular spaces lying between the two cell types (Fig. 6).

The light cell is always separated from the haemolymph by at least a thin overlay of dark cell cytoplasm. Communication with the haemolymph is, however, maintained by way of the sinusoids that penetrate the dark cell as described in the preceding paragraph.

Microtubules are found in both cell types, but they are more highly organized in the dark cell. The smooth form of the endoplasmic reticulum was not identified with any certainty in either cell type. The rough form,

however, is present in both. The Golgi complexes are small and few in number in either cell type (Fig. 7).

The light cell, in contrast to the dark cell, is extremely difficult to preserve in fixation. Even the best preparations reveal little cytoplasmic background detail (Figs. 7 and 14). It is probably highly hydrated in its native state.

Both cell types have indentations of the cell membrane at the cuticular surface. In the dark cell the cytoplasm is compartmentalized into tubular,

Fig. 7. Detail of light cytoplasm. Plane of section is parasagittal to one of the dark cell flanges, the broadened base being visible at the bottom of the photograph. Note the almost complete lack of cytoplasmic background material in the light cell as contrasted to the dark cell. Also, note the orderly distribution of strands of rough ER in the light cytoplasmic elements of the interdigitations. One of the infrequent Golgi systems can be seen (arrow). ×11,200.

regularly arranged, foot-like processes (Figs. 4 and 11). In the light cell they are more irregular in shape and depth (Fig. 6). These indentations are probably associated at least in part with the secretion of the cuticle.

II. Light Cell and Dark Cell Interdigitations

The cellular interdigitations are complicated but characteristic. The stellate dark cells surround and lap over the more cuboidal light cells. Extensive interdigitation occurs except where the cells are separated by the dense double membrane which starts at the cuticular border and extends inward between the two cell types (Fig. 4). The cytoplasmic projections

of the dark cell are in the form of thin sheets (Figs. 4 and 7) that penetrate into all parts of the light cell except for a zone about the nucleus. The sheets are rather evenly spaced and frequently anastomose to form a reticular structure resembling in some instances a honey-comb (Figs. 3 and 14).

Thus, the extensions of the dark cell partition the light cell into complementary interdigitations that are significantly larger in cross-sectional area

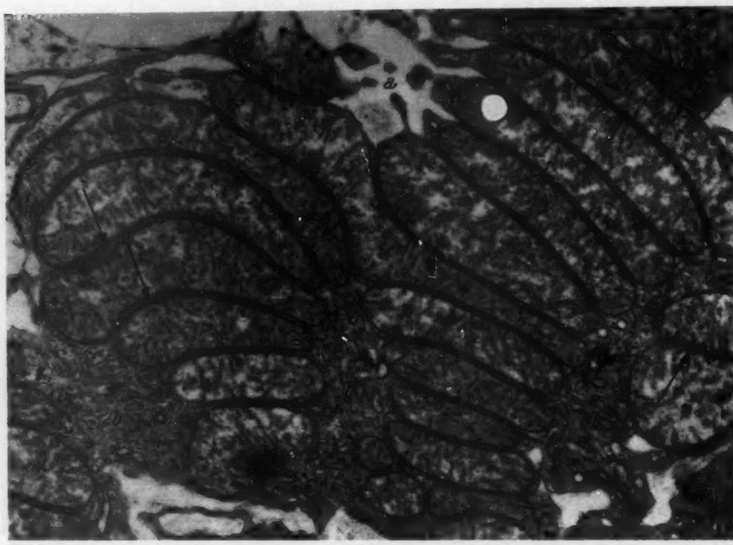


Fig. 8. Detail of mitochondrial pump system and canalicular associations. Plane of section is parasagittal to a dark cell flange. Haemolymph surface is out of the figure at the top. The lamellar spaces of the pumps communicate with the sinusoidal system of the dark cell (*a*) and continue into the body of the dark cell as canaliculi to intertwine with the rough ER (*b*). Sharp profiles indicate right angle sections of the pancake-like mitochondria. Blurred profiles indicate less than right angle section (arrows) and the broader dimension of the mitochondria becomes more evident in the latter case (see Fig. 4 for another example). ×15,400.

than of the dark cell projections (Fig. 3). In good preparations, almost all of the interdigitating extensions of the light cell exhibit a centrally located strand of rough endoplasmic reticulum (ER) (Figs. 7 and 14). The extensions of the dark cell also possess rough ER but it is not as plentiful and does not exhibit so consistent a pattern.

The dark cell interdigitating extensions are noteworthy for the large number of mitochondria dispersed throughout their cytoplasm. The diameter of the mitochondria is several times the thickness of the extensions, giving these dark cell projections a beaded appearance (Fig. 3).

Where the mitochondria bulge against the limiting cell membrane, the background cytoplasm virtually disappears, permitting a very close associa-

tion of the mitochondrial membranes and the two membranes of the interdigitating cells (Fig. 14). The intimacy of the membrane association, however, does not equal that described for mitochondrial pump in the next section.

There are very few mitochondria in the light cell extensions. In rough estimate, there are 15–20 dark cell mitochondria in the interdigitating area for every light cell mitochondrion (Figs. 3 and 14).

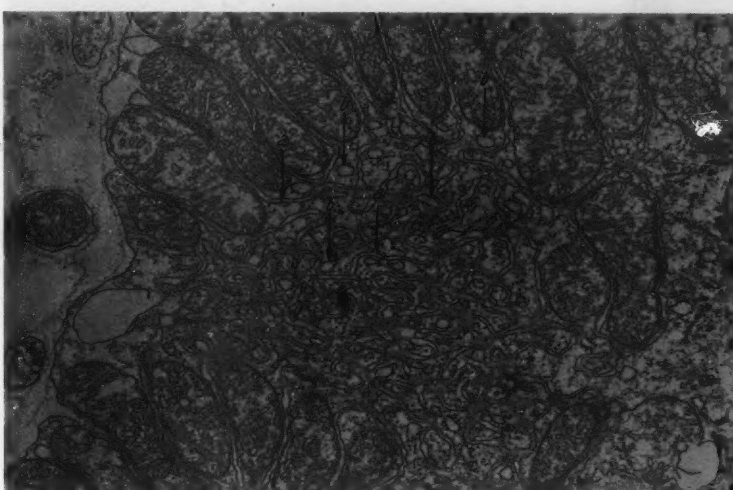


Fig. 9. Detail of rough ER and canalicular intertwining. Orientation of section same as in Fig. 8 except the plasma haemolymph surface of the cell is toward the left. This is a poor fixation (hypotonic) but it admirably illustrates the involved association of canalicular spaces (*a*) and rough ER spaces (*b*) adjacent to the mitochondrial pumps in the dark cell cytoplasm. Refer to Figs. 8 and 10 for more normal fixation. ×18,900.

III. Mitochondrial Pumps

Mitochondrial pumps radiate from the central axis of the dark cell close to the plasma surface. These are flattened, pancake-like mitochondria aligned in stacks with a thin lamellar space (and associated membranes) between each adjacent pair (Figs. 2, 8 and 10). The lamellar membranes are continuous with the sinusoidal membranes in one direction and canalicular membranes in the other direction.

The mitochondrial pumps are found in the dark cell exclusively. It is not unusual to see 10 to 15 mitochondria in such an association and as many as 26 have been counted in one grid opening. Considering the stellate nature of the dark cell and the limitations inherent in surveying thin sections, the true numerical limit of mitochondria in one association must be much higher. In any event, the number undoubtedly varies and the size of any one cluster is probably of no significance *per se*.

Within a mitochondrial pump system the limiting membranes lie in close parallel array with constant spacing (Fig. 10). The lamellar space is 150 to 200 angstroms in width. The lamellar membranes are about 80 to 100 angstroms thick. They are difficult to measure with accuracy because a light

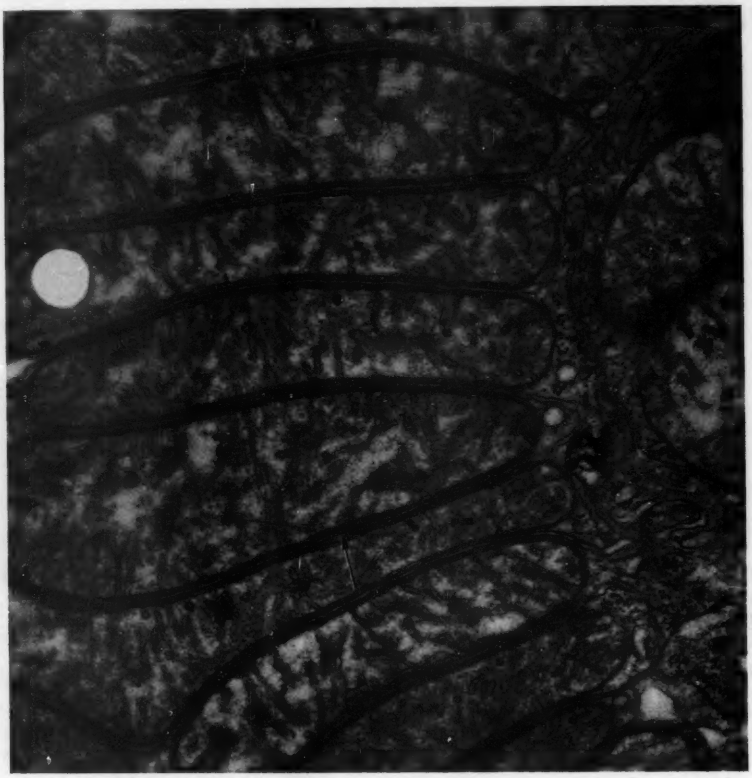


Fig. 10. Detail of mitochondrial pump. Photograph of same cell areas as in Fig. 8 but at higher magnification. Note the extremely small distance between the membranes both in regard to the lamellar space itself and in regard to the mitochondrial membrane association with the lamellar membrane. The arrow indicates one of the rare instances when a discernable amount of cytoplasm is seen between the lamellar membrane and mitochondrial membrane within a pump system. ×37,000.

fuzz seems to project from the membranes into or across the lumen. The spacing between the lamellar membrane and the envelope membrane of the mitochondrion is surprisingly regular and is about 100 to 150 angstroms, providing minimal diffusion distances between the mitochondrion and the lamellar membranes. The mitochondrial membrane is about 50 to 70 angstroms in thickness.

In addition to close apposition of the membrane systems, the mitochondria of the mitochondrial pump system are flattened in a pancake-like fashion.

Although the thickness of the mitochondria is relatively constant (Fig. 8) the diameter of individual "pancakes" probably varies. Furthermore, the greater dimensions (diameters) may not all lie in the same plane. The mitochondria may curve (Fig. 8) or twist (Fig. 4). In the latter case some idea of

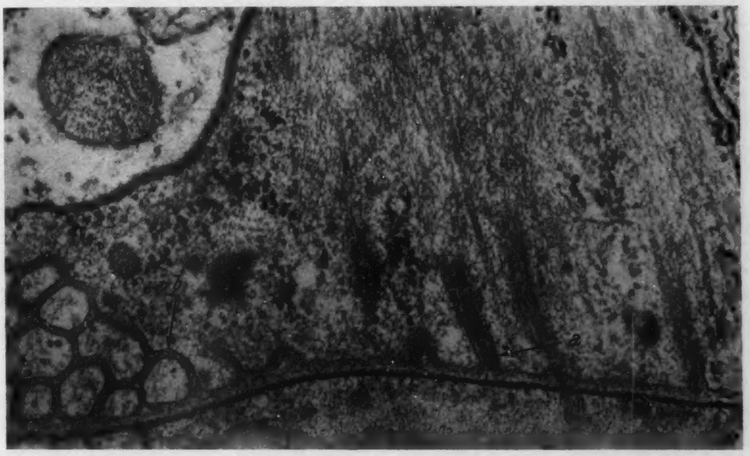


Fig. 11. Anchor fibers. A longitudinal, slightly tangential section of an anchor fiber (*a*) showing indentation of cell membrane about the fiber. Refer to Fig. 13 for a cross section at this level. Basal indentations of the dark cell can be seen at left (*b*). Note the roughly tubular configuration of the foot-like processes and that the intermembrane spaces are filled with granular material having the same texture as the layer between the cell membrane and cuticle (cuticle precursor?). ×48,300.

the lateral dimension of the mitochondria can be gained. In unusual cases the flattened mitochondria may nest within each other like a stack of cups; in which case, a section across the "cups" will show concentric rings of mitochondria (Copeland 1966).

As a corollary to the circumstance of the flattened form of the mitochondria, the lamellar spaces between the mitochondria are never seen as tubular spaces but always appear as clefts when viewed in cross section.

IV. Sinusoidal Spaces

Sinusoids are found exclusively in the dark cells. Invaginations of the cell surface exposed to the haemolymph may communicate directly with the mitochondrial pumps (Fig. 6) or develop into a spongy reticulum of sinusoids that in turn may communicate with either the mitochondrial pumps (Figs. 4 and 8) or the intercellular spaces located between the light and dark cells (Fig. 6). The continuations of the sinusoidal membranes

involved in the mitochondrial pumps (lamellar membranes) in turn connect to the canalicular membranes which intermesh with a network of rough ER located in the core of the dark cell (Figs. 8, 9 and 10).

V. Intercellular Spaces

The double membranes between the interdigitations are not always closely apposed and intercellular spacings occur (Figs. 4, 6 and 14). These seem to communicate freely with the sinusoidal spaces of the dark cell and, in turn, with the haemolymph (Fig. 6).

VI. Dense Double Membrane (Septate Desmosome)

The double membrane separating the bodies of the light and dark cells is filled with dense, homogeneous material (Fig. 4). At the cuticular end of the cell union, the membranes are connected to one of the basal indentations by a simple, compact desmosome (zonula occludens). Under favorable circumstances (usually in oblique section) a faint periodicity can be seen in the dense material. Between the apposed plasma membranes more removed from the cuticle, where they turn out onto the first interdigitating extension of the dark cell, the dense material exhibits a more and more widely spaced perodicity characteristic of septate desmosomes (Fig. 5).

VII. Microtubules, Microfibrils and Anchor Fibers

Microtubules are found in both cell types. In the light cell they are present in the prolongations which interdigitate with the dark cell (Fig. 14). They are not as numerous as those in dark cell but it is possible that many are lost because of the usually poor fixation of the light cell. In the main, they are oriented parallel to the long axes of the prolongations.

Microtubules are found in great numbers in the dark cell, particularly in the body of the cell. Here, whole areas are given over to bundles of microtubules paralleling the long axis of this part of the cell (Fig. 15). The bundles also show microfibrils which run parallel to the microtubules. The terminus of either the microtubules or microfibrils has not been determined.

Dense, rod-like fibers may be found at the base of the dark cells (Figs. 11, 12 and 13). They indent the cell membrane for a short distance and then fade into the microfibril-microtubule complex in a manner not yet resolved (Fig. 13). The same fibers also penetrate the cuticle and taper gradually until they disappear. They appear to anchor the dark cell to the cuticle much in the same way that fibers connect muscles and tendons.

Discussion

No approach to the study of osmoregulation in *Artemia* can now be made without first referring to the four definitive publications by Croghan (1958 a, b, c, d). Through a variety of experimental procedures he came to a number of general conclusions. For example, *Artemia* can live indefinitely in salinities from that of 10% sea water (3.5‰ total salinity) up to crystalizing brine (approximately 600‰ salinity). They drink freely

in all concentrations of water. Salt is actively transported from the gut into haemolymph. The haemolymph thus is always hypertonic to the gut and therefore obtains water passively from the gut. Excess salt is removed from the haemolymph by secretion from the first ten pairs of metepipodite

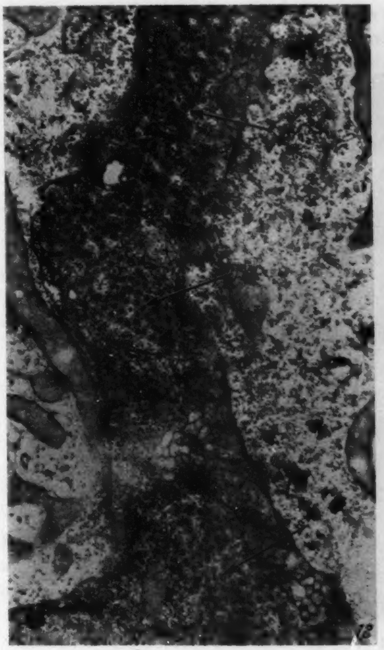


Fig. 12.

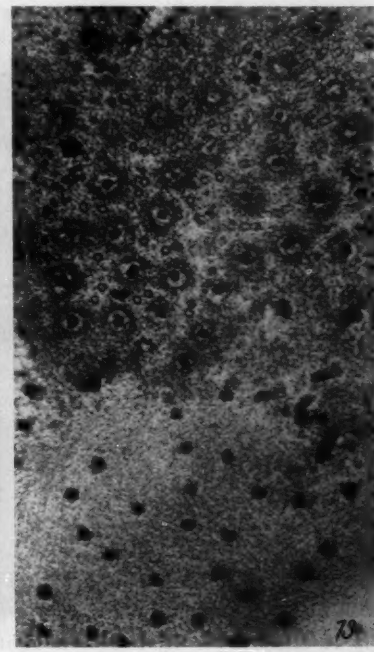


Fig. 13.

Fig. 12. Dark cell in a section parallel and close to the cuticle. Note the numerous and closely packed distribution of anchor fibers (arrows) in the central axis of the dark cell flange. Refer to Figs. 11 and 13 for details. ×9,400.

Fig. 13. Detail of anchor fibers and microtubules (dark cell). This section is slightly oblique to the plane of the cuticle. At the bottom is the cuticle with circular, dense cross sections of the anchor fibers. At the top are cross sections of anchor fibers with infoldings of the cell membrane surrounding them. Microtubules are also visible. ×50,400.

pads, the eleventh pair being nonfunctional. In about 9‰ salinity the osmotic pressure of the external medium and that of the haemolymph are equal. Below that salinity the haemolymph is hypertonic and above that salinity the haemolymph is hypotonic. The osmotic pressure of the haemolymph increases slightly with the increasing concentration of the sea water. However, this type of accomodation is limited to a maximum of about 27‰ equivalent salinity of the haemolymph when the animal is in

saturated brine (about 600‰ salinity). *Artemia,* therefore, has an efficient mechanism for osmoregulation.

We made no attempt to select any particular metepipodite pad for sectioning, but those near the middle of the row were usually larger and easier to cut open during fixation. At the histological level they look identical (with the exception of the nonfunctioning eleventh pair which we have not studied).

Fig. 14. Microtubules (light cell). The light cells are highly hydrated and extremely hard to fix. Occasional microtubules are seen (arrows) and are probably more numerous in the living tissue. This photo also depicts in good fashion the interdigitation of light and dark cell extensions. Note the intercellular spaces (*a*). Only one identifiable light cell mitochondrion is seen in this photograph (*b*). ×21,000.

Correlation of static morphology with physiological functions is difficult at best. One of the more significant interpretations can be made when the morphology changes with a change in physiological acitivity. This can be seen in *Artemia.* Animals which we raised in dilute sea water (10‰ salinity) isotonic with body fluids have metepipodites of normal size but the epithelial lining of the segments is quite degenerate, thin, and possesses few mitochondria. In contrast, the animals which we raised in triple strength sea water have in their metepipodites a thick epithelium with many mitochondria plus the cellular complexities described above. Thus, the cells of the segment hypertrophy and increase their cellular complexity under conditions of increased external salinity.

Equally significant is the occurrence of mitochondrial pumps in large numbers. This complex and intimate association of mitochondria and mem-

branes derived from the cell surface has been described only in one other tissue, the anal "gills" or papillae of mosquito larvae (C o p e l a n d 1964)[2]. It is interesting indeed that this system should be found in two unrelated animals living in opposite extremes of tonicity (0.009 to 105.0‰ total salinity). Although one animal is absorbing (mosquito larva) and the other secreting (*Artemia*), they both have in common the problem of moving salts metabolically against a steep gradient.

The mitochondrial pump is a highly diffrentiated and unique system. Most noteworthy is the constant width of the lamellar spaces (150 to

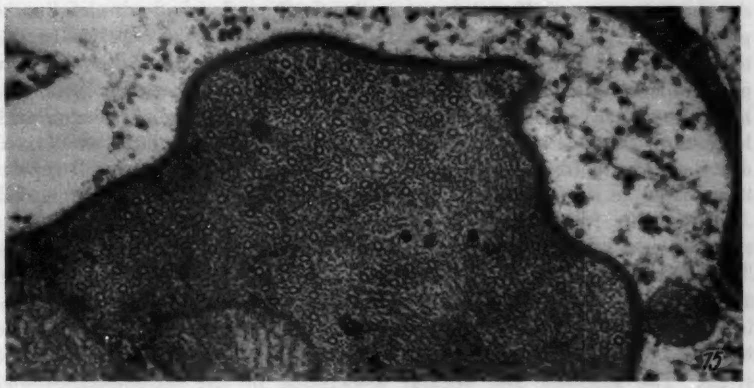


Fig. 15. Detail of microtubules and microfibrils (dark cell). This is a cross section of a dark cell midway between cuticular surface and haemolymph surface. Whole areas of the cell are given over to closely packed microtubules and intervening microfibrils. ×53,200.

200 angstroms) and of the gap between the lamellar membrane and the outer mitochondrial membrane (100 to 150 angstroms). Such constant spacing provides minimal diffusion distances and suggests the existence of a physical binding or perhaps a molecular attraction between the membranes.

The inner membrane of the mitochondria, which carries the cristae, lies close to the outer membrane of the mitochondria but the spacing does not show the same close tolerance exhibited by the distance between the mitochondrial and lamellar membranes. In poor fixations it is the first membrane to show displacement (Fig. 9). The spacing between the mitochondrial membrane and the lamellar membrane stays within narrow limits and is apparently determined by a tenacious association. As noted above, diffusion distances between the membranes are extremely short.

Another unique feature of the mitochondrial pump system as observed both in *Culex* larve and in *Artemia* is the flattened, or pancake, shape of

[2] Since this paper was submitted, a description of mitochondrial pumps in the rectal papillae of the blow fly has been made by G u p t a and B e r r i d g e, 1966, J. Cell. Biol. 29, 376.

the mitochondria. This distortion affords a much larger surface area for the intimate asociation with the lamellar membranes. The only analogous configuration, of which I am aware, is the observation in the synaptic bodies of the rod cells in the retina of the cat of as many as 7 mitochondria aligned surface to surface with 125 angstrom spacings (Pease 1962). There are two major differences in the analogy. In the mitochondrial system of the cat there are no intervening lamellar membranes and lumen. In the mitochondrial system of the brine shrimp there is no beading of the mitochondrial envelope membrane with a 160 angstrom periodicity as observed in the cat.

There is a possibility that a mitochondrial pump system exists in the pseudobranch gland of teleosts (Copeland and Dalton 1959). In this instance, mitochondria of normal shape (i.e. rod-shaped) are arranged in parallel rows with endoplasmic tubules packed closely between them, also in parallel fashion. Although the function of the glandular pseudobranch is unknown, it is interesting that the pseudobranch structure is derived morphologically from a hemibranch and the specific glandular cell type is homologous to the "chloride cell" of the gill, a presumed ion transport cell.

No proven function for the mitochondrial pump can be stated at this time. Physiologists may object to describing the mitochondrial system as a "pump" when no pumping action has been demonstrated. The term is used as descriptive of function inferred by strong indirect evidence. The system is described only in the salt mobilizing organs of two widely diverse invertebrates, both forms capable of existing under extremes of osmotic stress. The intimate and regular association of the mitochondrial and lamellar membranes would afford energy for efficient membrane transport through the lamellar membrane in the usually accepted sense.

However, the close approximation of the mitochondrial and lamellar membranes (100 to 150 angstroms) may limit diffusion between the cytoplasm of the cell and the internal (cytoplasmic) surface of the lamellar membrane. The narrow dimensions of the lamellar space (150 to 200 angstroms) might afford a more novel interpretation in that material might be moved selectively through the lamellar lumen by molecular forces resident in the lamellar membrane and energized by the mitochondria. Once stated, it is obvious that the same reasoning could be applied equally well to the space between the mitochondrial and lamellar membranes.

I am not completely happy with "mitochondrial pump" as the descriptive term for the system reported earlier in *Culex* and reported now in *Artemia*. Athough there is strong inferential reason to believe that the system is involved in metabolic transport, it is not intended to imply that the mitochondria necessarily act as the pumps. A condensed definition could be stated as follows: "The mitochondrial pump is a metabolically linked ion pump located in the cell plasma membranes intimately associated with the mitochondrial membranes at a distance of several hundred angstroms or less."

The possibility that the mitochondria may possess the site of active transport in their own membranes is not to be completely excluded. How-

ever, physiological investigations of metabolic ion transport systems for osmoregulation have in general localized the metabolic activity in the plasma membranes (or their continuations) of cells rather than in the membranes of some internal organelle such as the mitochondrion.

There are several marked differences between the absorbing epithelium in *Culex* larvae (Copeland 1964) and the secreting epithelium in *Artemia.* In the former animal, the lamellar space of the mitochondrial pumps may terminate at the edge of the pump in a bulging rim or continue into the general meshwork of the sinusoids. In the latter animal, terminal spaces are rarely seen and a predominance of the lamellar continuations extend into the body of the dark cell as canaliculi, there to intermesh with elements of the rough ER.

Perhaps the most noteworthy difference between the two animals is the existence of two distinct cell types in the *Artemia* tissue as compared to one type in *Culex.* The complicated and characteristic interdigitations of the alternating pattern of light and dark cells suggest that a functional relationship is involved. The light cells have a regular pattern of rough ER that could serve a transfer function. Indeed, if the rough ER of the dark cell extensions were as regularly arranged as that of the light cell, one might hypothesize a countercurrent system at the cellular level. More plausible, however, is the possibility that water, and perhaps other material, could be attracted from the "highly hydrated" light cell cytoplasm to the very "concentrated" cytoplasm of the dark cell. In actuality the degree of electron density does not necessarily indicate the relative tonicity of the cytoplasms.

The almost exclusive concentration of mitochondria in the dark cell interdigitations may indicate a membrane transport system with the energetics limited to the dark cell membranes.

The possibility of a functional relationship between the light and dark cells is suggested by factors other than the unique interdigitation. The dark cell is the only cell of the two types that has direct contact with the circulating haemolymph. The light cell has indirect contact only through the intracellular canaliculi that penetrate the dark cell.

Of considerable interest is the dense membrane that separates the dark cell from the light cell at all points except where interdigitation occurs. No function is obvious for this very dense membrane though it does seem to be unique to the salt secreting tissue of *Artemia.* The periodicity to be observed at one terminus of the membrane and, in favorable sections, more faintly throughout the dense portions suggests that a modified septate desmosome is involved. The cross linkages originally described for septate desmosomes (Wood 1959) in this instance are of a size that obliterates the intervening spaces, resulting in a "solid" septate desmosome. It is hypothesized by Wood (1959) that the septate desmosome impeded or prevented movement of water between the epithelical cells of Hydra. In *Artemia,* it is possible that the dense membrane prevents ready access of water or other materials from the one cell type to the other, except in the interdigitations where the dark membrane does not occur.

There is also the possibility that the dense membrane or junctor may be a site of lowered resistance to passage of ions. If the dense parts of the septate junctors discussed by Loewenstein and Kanno (1964) allow free passage of ions, then the *Artemia* dense membrane is highly specialized to allow intercellular movement of ions. Contrariwise, if the movement of ions is limited to the light areas of the septate junctor (with the dense areas acting as lateral barriers), then the *Artemia* membrane would be highly impervious to ion movement.

The basal indentations found at the cuticular border of both cell types are probably linked to the function of secretion of the cuticle. No mitochondria are associated with them, nor is there any evidence of pinocytosis or other indication of membrane transport *per se.* Similar folds are to be observed adjacent to the cuticle in other branchial elements of *Artemia.*

The rod like "anchor fibers" at the cuticular base of the dark cells are observed in other dermal elements of *Artemia* and probably serve as mechanical attachment between dermis and cuticle, similar to the fibers in the myo-tendonal junctions.

Microtubules and associated microfibrils are found also in other branchial segments of the *Artemia* phyllopods, but are not usually as numerous and densely packed as in the dark cell of the metepipodite. Isolated microtubules are also found in the light cell and their pattern in the latter case is not completely certain because of fixation difficulties.

Microtubules have been described in a wide range of tissues but the term "microtubule" has not been used in a defined or collective sense until comparatively recently (Slautterbach 1963, Ledbetter and Porter 1963). Just as the original concept of "unit membrane" now has its variations, eventually microtubules will probably fall into several categories. For example, Slautterback (1963) in his review of observed occurrences suggests that microtubules of about 270 angstrom diameter may serve for elastic or structural support of the cytoarchitecture, and those in the 120 to 200 angstrom range may serve metabolic functions with ion transport and water movement being strong possibilities. Unfortunate to interpretation in this instance, the size of the microtubules in the *Artemia* metepipodite tissue approximate 270 angstroms in total diameter. Nevertheless, the large numbers of microtubules and associated microfibrils found in the dark cell suggest that they may be related to the functioning of that cell.

The complexity of the unique light and dark cell relationship plus the presence of mitochondrial pumps leaves little doubt but that *Artemia* possesses a highly specialized tissue for the secretion of salt. Which cell type serves as the final pathway for the release of salt to the external environment is not certain. However, it would appear likely that the dark cell is responsible. The dark cell has dense cytoplasm, the tonicity of which may equal or exceed that of the environment so that salt, perhaps in chemically bound form, could be released to the externum without undue osmotic stress to the cell.

If it is assumed that salt is moved metabolically and that water moves in association with the salt (Diamond 1962 and 1965), then it can be

hypothesized that the mitochondrial pumps move salt and accompanying water. Then the light-dark cell interdigitations may in some fashion be involved in a compensatory shift of water alone through osmotic differences rather than metabolic forces.

Histochemical approaches are now being explored in an effort to obtain better insight regarding the functioning of this unusual tissue. Its complexity defies analysis of function on a purely morphological basis.

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Observations on the Fine Structure of the Oat Coleoptile

I. The Epidermal Cells of the Extreme Apex

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With 26 Figures

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Introduction

The relationship between structure and function is a central quest in modern biology. The study of function is inevitably the study of a specific object and it would be difficult to overestimate the contribution of the coleoptile to the fields of plant physiology and plant biochemistry. Most biologists are familiar with the series of experiments which began with the work of the Darwins in 1880 and culminated in the 1930's with the isolation of auxin, the first plant hormone. The marked sensitivity of the coleoptile to auxin made it a favored object for bioassaying auxins extracted from other plants (Went and Thimann 1937, Ch. III). In 1936, Avery and Burkholder showed that cell division ceases in oat coleoptile parenchyma when the organ attains a length of about 10 mm: the bulk of the extension growth of this structure is therefore uncomplicated by cell division. Segments from such "mature" coleoptiles (25 mm) have been used extensively to test a vast array of compounds for activity as cell elongation substances. The long cells of the outer epidermis show a well-developed cytoplasmic streaming (Bottelier 1935) and the effect of auxin on this process has been studied by Sweeney and Thimann (1938); Thimann and Sweeney (1942). In recent years, these same cells have been used to make very precise analyses of the kinetics of auxin-induced growth (Ray and Ruesink 1962). Since large numbers of relatively uniform

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coleoptile-segments may be obtained from plants grown in the laboratory under defined experimental conditions, the coleoptile has been a favorite tissue for the study of the biochemical and biophysical effects of auxin (Bonner 1933; Noodén and Thimann 1965).

In turn, the behavior of the organ which has served so admirably as a test object in the study of plant growth has itself been investigated intensively. It was the phototropic sensitivity of the coleoptile which led to the discovery of auxin and perhaps it is only fitting that phototropism, as it exists in higher plants, is best understood in the coleoptile (Curry 1957; Thimann and Curry 1960; Briggs 1963; Thimann 1964). The Cholodny-Went theory of tropisms, formulated in the late 1920's, explained growth curvatures induced by light and gravity in terms of lateral transport of auxin. Although some reservations need to be made for the geotropic reactions of roots (see Konings 1964) most workers agree that the theory offers a satisfactory explanation of the behavior of the coleoptile, and probably of shoots in general. In this and other reactions, it is clear that the control of growth and tropism in the coleoptile is vested largely in the tip, the uppermost 1–2 mm. In particular, in the young seedling on its way to the soil surface, it is the uppermost part of the coleoptile which protects the shoot from mechanical damage, produces the growth-limiting supply of auxin (perhaps from a precursor which comes from the seed) and transports the auxin polarly to the growth zone beneath. It is the tip which is especially sensitive to blue light and reacts to a unilateral stimulus by the induction of a net lateral transport of auxin.

Considered together, these facts constitute an impressive list of physiological capacities. Yet the complementary aspect, namely the structure, has been relatively little studied, and in particular, surprisingly little is known of the structure of these apical cells.

Previous studies of the fine structure of the coleoptile have been concerned almost exclusively with the cell walls (Bayley et al. 1957; Böhmer 1958; Mühlethaler 1950; Setterfield 1957; Setterfield and Bayley 1958, 1959; Wardrop 1955, 1956; Wardrop and Cronshaw 1958). While these observations have yielded valuable information about the orientation of the fibrillar component of the wall and have posed some interesting questions about the mechanism of cell-wall growth, especially in the epidermal cells, the methods of the 50's were such that almost all the noncellulosic polysaccharides and the bulk of the cytoplasm were extracted or badly disorganized during the preparation of the specimen for electron microscopy. Subsequently, Arrigoni and Rossi (1962, 1964) and Wardrop and Foster (1964) have presented some observations based upon permanganate-fixed tissues and this work has been extended by Cronshaw (1965) and Cronshaw and Bouck (1965) who have studied some aspects of xylem differentiation in material fixed with glutaraldehyde/OsO_4. Both Thornton and Thimann (1964) and Cronshaw (1964) have also described the crystal-containing bodies of the parenchyma cells of the oat coleoptile.

Methods of specimen preparation for electron microscopy have improved

dramatically during the last 10 years; indeed, the methods described here have undergone an evolution during the time these studies have been in progress. This work began (in December 1962) because it seemed likely that an intensive investigation of the fine structure of this much-studied organ, using modern methods, might give some insight into its physiology. It became apparent quite quickly that fully effective electron microscopy of this organ demanded first an adequate understanding of its histology, and this is now available (O'Brien and Thimann 1965; Thimann and O'Brien 1965).

This paper is mainly concerned with the relatively short, heavy- walled cells of the outer epidermis of the extreme apex of the coleoptile; in addition, a few observations on the guard cells are included. The fine structure of these epidermal cells is of interest for several reasons. First, they belong to a class of cells of great importance to higher plants since epidermal cells carry out an array of regulative and protective functions (Esau 1965). In the coleoptile, these cells must also protect the organ from abrasion following normal germination beneath a layer of soil.

Secondly, epidermal cells are both robust and accessible, and these two properties have made them favorite objects for study. For example, the cells of the inner epidermis of the onion bulb have probably been examined by light microscopy and the techniques of vital staining more than any other cell type; much of our *in vivo* knowledge of plant cells is based on these cells. It is a great pity that, with the exception of the observations on the surfaces of plants (e.g., Juniper 1959; Skene 1963), most of our present understanding of these cells is based upon material which has been preserved in a rather poor condition. Knowledge of their fine structure is indeed meager and it is clear that these cells have posed serious problems of specimen preparation (see e.g., Bancher et al. 1960; Bolliger 1959, Drawert and Mix 1963; Frey-Wyssling and Mühlethaler 1965; Scott et al. 1958).

Thirdly, since the oat coleoptile may grow at a rate of about 1 mm/hr, the epidermal cells in the zone of rapid elongation must synthesize at least 35 μ^2 of wall surface per second: the fine structure of the cytoplasm/wall interface in such cells might well be expected to yield some information about the mechanism of wall synthesis. However, it seemed wise to examine first the very slowly growing cells of the tip of the coleoptile in order to distinguish more readily between those aspects of fine structure which function in growth and those which are simply correlated with the epidermal nature of the cell.

In addition, the methods of specimen preparation are given here in full, for two reasons. First, successful preparation procedures for cutinized parts of higher plants are not widely known. Secondly, few of the published papers on the fine structure of higher plant cells cite the methods in enough detail to allow another investigator to duplicate the work; commonly, important and even critical points (e.g., temperature and duration of fixation, speed of dehydration, period of infiltration, etc.) are omitted.

Materials and Methods

A. Preparation of Plant Material

Coleoptiles of *Avena sativa* L. var. "Victory," "Ajax," "Garry, "Rodney," and "Exeter," and of *Avena fatua* L., were grown to a length of 25 mm by the methods described in detail by O'Brien and Thimann 1965.

B. Fixation

(i) Phosphate buffered OsO_4: 2% OsO_4 in 0.1 M sodium phosphate buffer at pH 6.8 for 12–20 hours at room temperature.

(ii) Glutaraldehyde OsO_4: 1.5–5% glutaraldehyde solution (Sabatini et al. 1963) in 0.025 M sodium phosphate buffer at pH 6.8 at 0° C for 2 hours to overnight. Occasionally, tissues were fixed in 0.5–1.5% glutaraldehyde solution for 1–2 hours and then transferred to 5% for an extra 12–16 hours. Tissues were transferred through several rinses of 0.025 M buffer at 0° C to remove excess glutaraldehyde, with a minimum of 4 rinses in 4 hours, and occasionally were left overnight in the last rinse. Post-fixation was in 2% OsO_4 in 0.1 M sodium phosphate buffer at pH 6.8, either at room temperature for 3 hours, or overnight at 0° C.

Note: microtubules (Ledbetter and Porter 1963) are not always preserved in some cell-types if the fixation in glutaraldehyde is carried out at O°C. However, it was our experience that the mature cells of the coleoptile were very badly damaged if the fixation in glutaraldehyde was carried out at room temperature. No satisfactory solution to this dilemma has yet been devised. Secondly, great care must be taken to ensure that the glutaraldehyde used is free of impurities. If the stock solution forms a precipitate on dilution with buffer, or if it contains any appreciable content of acid, it should be re-distilled, or another fresh, pure batch obtained.

C. Dehydration

(i) Acetone-Water Series: In general, tissues were transferred directly to 30 % acetone in water at 0°C, without a wash after the osmium fixation. They were then moved through 50%, 70%, and 90% acetone in water to 100% acetone (two changes), with a minimum of 1 hours and a maximum of 16 hours per change, all at 0°C. Propylene oxide was added gradually to the tissue in acetone, and brought to a concentration of about 50% propylene oxide over a period of 8 hours. The tissue was transferred through two more changes of propylene oxide, allowed to come to room temperature, and given one further change at room temperature.

(ii) Methyl Cellosolve Series: Tissues post-fixed in osmium were given two 0.5 hour washes in 0.025 M sodium phosphate buffer at 0° C and transferred for a minimum of 6 hours to methyl cellosolve at 0° C. The specimens were transferred to a deep freeze at — 25° C, and given two further changes in methyl cellosolve. They were then transferred to ethanol (100%) at — 25° C and given two changes in 24 hours, then transferred to pure propylene oxide at — 25° C and given two changes in 24 hours. They were then allowed to come to room temperature in the propylene oxide and given one more change at room temperature. Later studies have suggested that the steps up to and including the ethanol transfers are just as satisfactory if carried out at 0° C. However, the transfer from either acetone or ethanol to propylene oxide is best done at — 25° C, because there is good reason to believe that this step is one of the most damaging of any in the dehydration schedule, and the damage is minimized if the temperature is low during the solvent exchange (Feder and O'Brien, unpublished).

D. Infiltration and Embedding

(i) The Resin Mixture: All experiments have used one of two mixtures, both devised by Dr. M. C. Ledbetter. One consists of Araldite 6005, 37.5%: Araldite RD-2, 12.5%: dodecenyl succinic anhydride (DDSA), 50%; these proportions are by volume. The other recipe consists of a 1 : 1 mixture of Araldite 6005 and DDSA, but the proportions are by weight. In general, large batches (2 lb. or more) were mixed up with a power stirrer, subdivided into 2 or 4 oz. lots in screw-capped jars, and held in a deep freeze. The wax-coated paperliner of the screw cap was removed and replaced with aluminium foil. Such jars were allowed to warm up to room temperature for at least 24 hours in a desiccator containing "Drierite" before being opened for use. This precaution prevented water from condensing on the cold resin mixture.

(ii) Infiltration: A sample of the resin mixture was made 0.5% in DMP-30 (acceleration catalyst—tridimethyl-aminomethylphenol) and mixed with an equal volume of propylene oxide. In early experiments, the tissue in propylene oxide was simply transferred to this mixture; this was abandoned when it was noticed that the tissue shrank markedly. In later work, the 1 : 1 mixture of resin in propylene oxide was added a few drops at a time to the tissue in propylene oxide until a concentration of about 15% resin in propylene oxide was reached. Enough of the 1 : 1 mixture was then added to ensure that the tissue would be covered by resin when all the propylene oxide was evaporated, and the vials were left in a fume-hood until all the propylene oxide was gone (usually overnight). The tissues were then transferred to fresh resin-mixture containing 0.5% DMP-30. This was prepared by adding the DMP-30 and resin mixture in the correct proportions, and thoroughly mixing them with a power stirrer for 3–5 minutes in a glass jar. The tissues were allowed to infiltrate in this mixture for a minimum of 4 days and on occasion for up to 10 days. These long infiltration times were essential for adequate penetration of the thick walls of the outer epidermis. Every 3–4 days, the tissues were transferred carefully (i.e., they were picked up on a flat wooden stick, not with forceps) to freshly prepared mixture, and at some time during this infiltration period, they were evacuated for 12–16 hours.

(iii) Embedding: For flat embedments, the tissue was transferred to fresh resin and accelerator (0.5–1.0%) mixture which was contained either in suitable "boats" made out of aluminum foil, or in the aluminum foil weighing dishes supplied by Fisher Scientific. For embedding in gelatin capsules, the following procedure (devised by Dr. M. C. Ledbetter) was used. The gelatin capsules were first "tipped" by polymerizing a drop or two of any epoxy resin mixture in the bottom of the capsule. Next, the specimen was introduced in just enough resin mixture to cover it, and oriented on the hard plug of plastic. The capsules were aspirated for about 1 hour and polymerized at 60° C until the plastic was firm, but not hard (usually 10–12 hours). The capsule was then filled with a resin mixture that contained 3% accelerator, and polymerized for an extra 1–4 days. This last step ensured an extremely hard shank which gave good support for the block during sectioning.

E. Sectioning and Staining

Sections were cut with a diamond knife on a Huxley ultramicrotome and those which showed silver/gold to grey (essentially zero order) interference colors after flattening with xylene vapor were picked up on freshly-cleaned copper grids (200 or 400 mesh). The sections were "stained" on the grid, usually with uranyl acetate for 15 minutes (saturated aqueous solution, freshly filtered before use; Watson 1958) followed by lead citrate for 5–10 minutes (Reynolds 1963).

Precipitates of stain on the section were minimized by

(i) wetting the grid with distilled water before introducing it into the uranyl acetate solution;

(ii) rinsing the grid carefully with water between the uranyl acetate and lead citrate stains;

(iii) carrying out the lead citrate stain in an atmosphere of low CO_2-tension. This was achieved by staining the sections in a closed petri dish which contained a reservoir of sodium hydroxide pellets. After staining, the sections were rinsed briefly in distilled water and dried by touching the grids to the edge of a filter paper disc. When dry, the sections were coated with a thin (30–70 Å) layer of carbon to minimize "drift" in the microscope and the sections were examined in an RCA EMU 3 D at 50 Kv, or in a Siemens Elmiskop I at 80 Kv.

F. Light Microscopy

Sections for light microscopy of tissue embedded in Araldite were cut 0.25–0.5 μ thick, flattened with xylene vapor, picked up in a drop of water by means of a fine, clean copper loop, and dried at 60° C in a drop of Seitz-filtered distilled water on a slide. Such sections were stained for survey work in a mixture of 1% methylene blue containing 1% borax, and 1% Azure II, as outlined by Richardson et al. (1960). This procedure does not give permanent mounts as the sections may fade in as little as a week. Material embedded in Araldite was also stained with the PAS procedure (see Jensen 1962) but the hydrolysis in periodic acid was carried out for 30 minutes. Toluidine blue 0 (O'Brien et al. 1964) will also stain materials embedded in epoxy resins but the time of staining must be increased to several hours (see also Jacobson et al. 1963). Acid dyes (e.g., acid fuchsin, fast green) do not usually stain osmium-treated tissues embedded in epoxy resins. Presumably the amino groups with which these dyes react have been complexed either with the OsO_4 during fixation, or with the epoxide group of the resin.

G. Metal-Shadowing of Araldite-embedded Sections

A full analysis of this technique will be published at a later date (Maser, O'Brien and McCully, in preparation). In brief, grids of sections were shadowed under vacuum with platinum at an angle of 2 : 1, using standard technique. The resulting sections exhibit considerable surface relief when examined in the microscope even though the embedding material has not been removed. Such sections form a useful adjunct to ordinary sections because it is often possible to distinguish the fibrillar structure of the cell-wall.

Observations and Results

A. The Cell-wall

Fig. 1 shows part of a transverse section of the epidermis (TS; 0.25 μ thick) taken at a level about 0.5 mm from the apex of the coleoptile. The tissue was fixed in glutaraldehyde/OsO_4, embedded in Araldite, and stained with methylene blue/azure II. The outer wall shows several layers. A cuticle covers the surface and cutin cystoliths (see Fritz 1937) occur at various levels in the wall. An intensely stained layer, *pl*, separates the cuticle from the wide, weakly-stained layer, *ol*, which constitutes the bulk of the thickness of the wall. In general, the inner region of the wall shows two layers of somewhat variable thickness, the outer one of which tends

to stain more darkly than the inner. The degree of this differentiation of the inner part of the wall into two layers varies from cell to cell around the epidermis at any one level of transection and is correlated with the fine structure of the layer (see below).

Fig. 2 shows the same section as that illustrated in Fig. 1 and at the same magnification, stained with the PAS procedure. Again, the wall shows distinct layers; the cuticle and cutin cystoliths are, of course, unstained and the intensely-stained layer (*pl*) of Fig. 1 is much less obivous. The wide layer of the wall (*ol*; strongly PAS-positive) seems to be continuous with the radial walls; note the fine strands of PAS-positive material which penetrate the cuticle at several points (arrow).

Fig. 3, an electron micrograph of a section adjacent to that shown in Fig. 2 (outlined area) shows that the EM reveals the same gross structure of the wall. The cuticle and cutin cystoliths are both intensely osmiophilic but at this magnification, the osmiophilia obscures the nature of the material which is responsible for the intensely-stained layer, *pl*, of Fig. 1. The wide layer, *ol*, of the outer wall appears to be continuous with the middle region of the radial walls, and is sharply differentiated from the more electron-opaque inner layer. The electron-opaque inner region in Fig. 3 corresponds closely to the inner layer (less PAS-positive) of Fig. 2, and again shows a faint differentiation into two or more layers. The structure os these layers of wall is treated in more detail below.

(i) The cuticle, cutin cystoliths, and the outer layer of the wall. The fine structure of these regions of the wall is illustrated in Figs. 4 and 5. Because the osmiophilia of the matrix of the cuticle varies considerably among the different varieties of oats, the structure of ***Avena fatua,*** in which the matrix has a very low osmiophilia, is illustrated here. It is to be understood, however, that this is the only respect in which this layer differs from that seen in any of the varieties of ***Avena sativa*** which have been examined. The bulk of the cuticle and cutin cystoliths consists of an apparently structureless matrix through which ramifies a reticulum of electron-dense fibrillar material. This fibrillar material ranges in thickness from about 500 Å down to the limit of resolution. The fibrils extend almost all the way through the amorphous phase but stop just short of the surface of the cuticle. At the edges of cutin cystoliths and at the base of the cuticle the reticulate nature of this material is more obvious, especially when seen in a plane tangential to the surface of the layer (Fig. 5, asterisk). Clearly, it is this region, especially enriched in the material which makes up the reticulum, which stains so intensely at the base of the cuticle and margins of the cystoliths (*pl* in Fig. 1).

The outer wide layer of the wall, *ol*, shows only a faint layering at low magnification (Fig. 3), but at higher magnification (Fig. 4), one can distinguish within this layer a finely-textured mosaic of at least two components. One of these components consists of electron-dense granules and fibrils 30–80 Å in diameter; the other component is electron transparent but could be described as an array of fibrils and granules of somewhat larger dimension (80–200 Å). At the base of the cuticle, the electron-dense

phase of the outer layer of the wall merges imperceptibly with the fibrillar reticulum of the cuticle. Since little is known of the chemistry which underlies the staining of cell-wall components by the heavy metals used in electron microscopy, and since staining reactions of basic dyes are unreliable in osmium-treated tissues, it was not possible to establish the nature of the fibrillar material from these sections. However, carefully controlled staining of 1μ sections of walls in tissues fixed in acrolein and embedded in glycol methacrylate (see O'Brien and Thimann 1965) showed that polyuronides were present throughout the width of the wall, and were especially rich in the region which corresponds to the layer, *pl*, of Fig. 1. It seems reasonable to suggest that it is this polyuronide component of the wall which stains with the heavy metals and that polyuronides are a major component of the fibrillar reticulum.

(ii) The inner region of the wall. The features of the wall described above have proved to be remarkably constant in the different varieties of *Avena sativa* examined, and in *Avena fatua*, but the structure of the inner part of the wall is much more variable. Variation in the structure of the inner layers of the wall arises chiefly from the degree to which this region contains the multivesicular elements previously described (O'Brien 1965). All varieties so far examined lie between the extremes illustrated in Figs. 6 and 8. Fig. 6 shows the inner wall in var. "Rodney" in which no trace of these elements has ever been seen. Instead, the lamellations of the inner layer persist right up to the plasmalemma. At higher magnification (Fig. 7) it may be seen that this lamellated structure is due to layers of wall material of alternating electron density. Two dense layers appear to be joined to one another by a fibrillar mat which often imparts to the lamella a structure reminiscent of a herring bone. On the other hand, Fig. 8 shows the structure of this layer in var. "Ajax." In this variety, the lamellae of the outer part of the inner layer of the wall give place to a region in which the wall material is severely distorted. The distorting elements range from 0.02–0.3 μ in diameter. Even at high magnification no membrane has ever been detected around these structures although the margins of the elements are often more electron-dense than the nearby matrix (Fig. 9, arrows).

In some sections, many vesicles, and especially the larger ones, appear to contain material of low electron contrast (Fig. 10), but on other occasions they appear to be empty (Fig. 9). It is a curious fact that the lamellation of the wall often continues undisturbed in those parts of the inner layer

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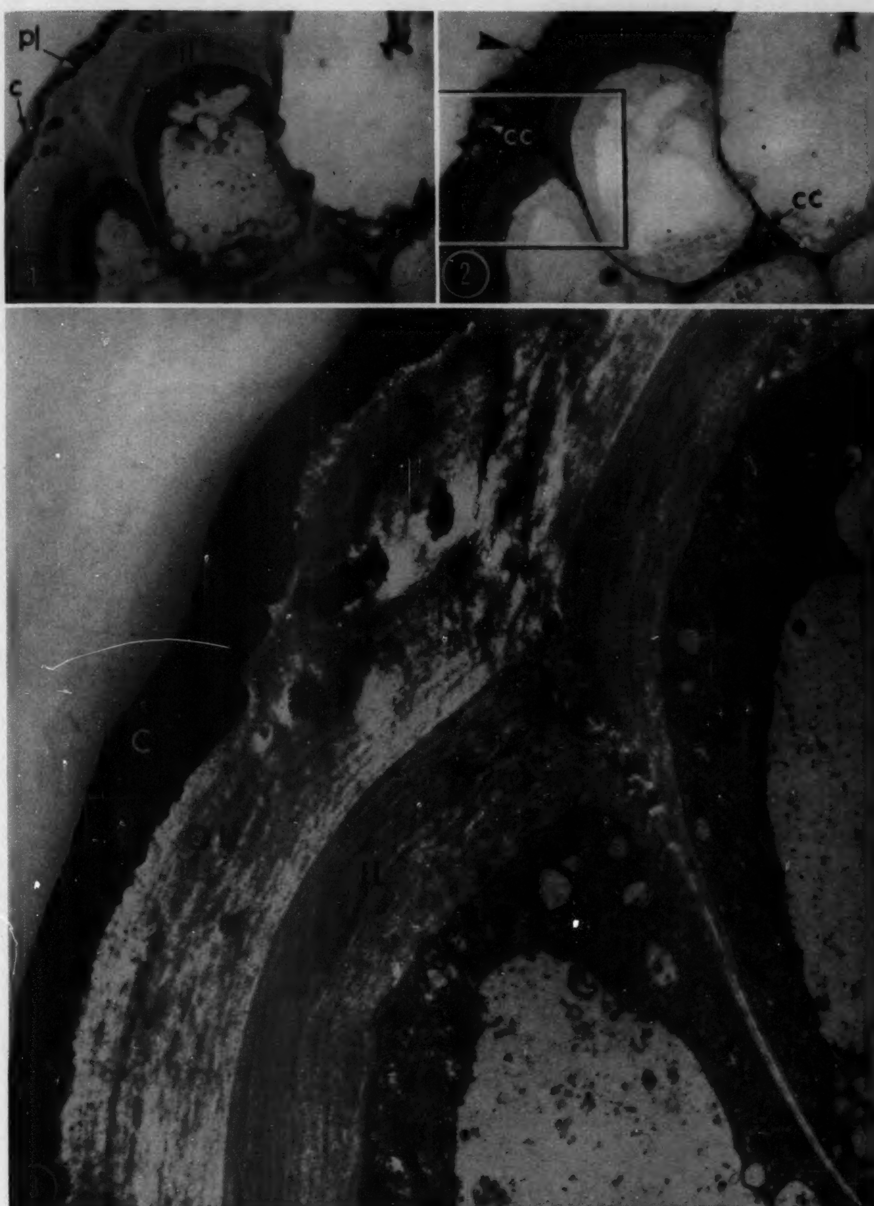
Fig. 1. Light micrograph of an 0.25 μ thick section (TS) of the outer epidermis of *Avena sativa* var. "Victory," about 0.5 mm from the apex of the coleoptile. Stained with methylene blue azure II. Note the cuticle and the three layers of the wall. ×1,320

Fig. 2. The same section as that shown in Fig. 1 but stained with the PAS reaction. Note the thin strands of material which penetrate into the cuticle (arrow) and the cutin cystoliths at the bottom of the radial walls. ×1,320.

Fig. 3. Electron micrograph of the region adjacent to that outlined in Fig. 2. The same gross features of the wall are discernible in the EM. ×5,750.



Figs. 1–3.

of the wall that are free of these elements, even in var. "Ajax" (Fig. 8, arrow). In *Avena sativa* var. "Victory" the structure is intermediate between the extremes shown by var. "Ajax" and var. "Rodney" (Fig. 12 and O'Brien 1965). To determine the distribution of these elements, sections from a 35 mm coleoptile of var. "Victory" were examined at various positions (1, 2, 8, 16, and 35 mm from the apex) and it was found that these structures occurred only in the uppermost 2 mm. The walls of mature coleoptile tips fixed only in phosphate-buffered OsO_4 show similar structures (O'Brien 1965). It seems reasonable to conclude that these elements reflect some irregularity in the deposition of this inner layer of the wall and a possible interpretation, which takes into account the variation seen in the different oat varieties, is offered in the discussion.

(iii) The anticlinal (radial) and inner tangential walls. Figs. 1 and 2 show that where two epidermal cells abut one another, the outer walls of the adjacent cells connect with the radial walls as a wedge- or fountain-shaped thickening.

In the EM, the thin parts of the radial and inner tangential walls do not show any pronounced lamellation, but the image of these walls after the normal double "staining" with uranyl acetate and lead citrate is quite variable. Usually, the compound middle lamella region is a little more electron-dense than the rest of the wall, but on occasion, either no distinction is evident or the contrast is reversed (cf., Figs. 3, 15, and 19). However, the wedge-shaped thickenings of the outer extensions of the radil walls do show a definite and reproducible fine structure. One may usually discern the wedge of material of low electron contrast which joins the middle region of the radial wall to the outer layer of the outer wall. Both regions show essentially the same fine structure (Figs. 3 and 11) and in shadowed preparations, this region is free of lamellae (Fig. 13). The lamellae of the inner layers of the outer walls continue into these corner thickenings but decrease progressively in thickness (Fig. 12). This continuity of the lamellae of the outer wall with the corner thickenings is particularly evident in shadowed sections (Fig. 13).

These corner thickenings often contain multivesicular elements also and the frequency and arrangement of these elements in these thickenings depends upon the variety of oat in much the same way as it does in the inner layer of the outer wall (Fig. 11). Multivesicular elements have never been detected in the radial wall except close to these thickenings.

B. The Cytoplasm

Those gross cytological features of these cells which are discernible with the light microscope (O'Brien and Thimann 1965) are at once confirmed and explained in the ER. The cytoplasm stains readily both with acidic and basic dyes, and the basophilia is clearly related to the wealth of cyto-

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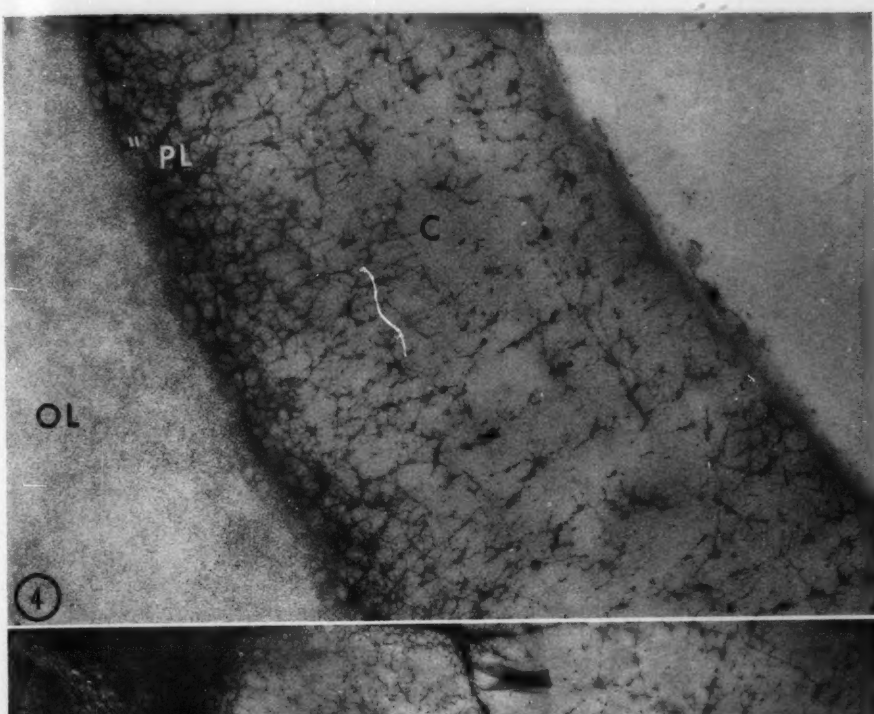
Fig. 4. The fibrillar reticulum of the cuticle of *Avena fatua*. ×68,000.

Fig. 5. Grazing section through the base of several cutin cystoliths to reveal the reticulum (asterisk) which surrounds them. ×22,600.



Figs. 4 and 5.

plasmic ribosomes. These occur both free in the ground substance, and bound to the membranes of the endoplasmic reticulum (ER) (Figs. 9, 15, 16, and 21). The acidophilia may be attributed to the wealth of cytoplasmic ground substance, which is several times more electron dense in these cells than in the underlying parenchyma cells. Mitochondria are numerous with well-developed cristae and are rich in the small black granules which are believed to be the sites at which calcium salts are deposited (Figs. 15 and 19: see Lehninger 1964). Plastids are sparse and poorly developed (Figs. 3, 6, 11, 15, and 16). Usually they contain no grains of carbohydrate (very small ones are present occasionally) and develop only a few lamellae. No trace of grana has ever been seen. The plastid stroma contains only a few granules which could be interpreted as chloroplast ribosomes (Jacobsen et al. 1963; Gunning 1965a; 1965b). Both the plastids and the mitochondria do contain areas which resemble bacterial nucleoids (Fig. 15), and since there is now good evidence for the existence of DNA in both of these organelles (Swift 1965) it seems reasonable to suggest that these nucleoid-like areas may be DNA-rich regions (see also Gunning 1965a; Nass and Nass 1963). Dicyosomes occur quite frequently, and there is some evidence that they may be contributing material to the wall (see below).

Apart from these general observations, there are several other features in the fine structure of these cells that seem important. First, the appearance of the nucleus suggests that this is a highly differentiated cell-type, for a considerable fraction of the chromatin which is preserved by this fixation (glutaraldehyde/OsO_4) occurs as aggregates of varying size (Figs. 14 and 15). In sharp contrast to the situation in many differentiated animal cells (see Porter and Bonneville 1963 for examples), this aggregated chromatin (as seen in section) is not confined largely to a band around the margin of the nuclear envelope. Rather, the aggregates seen to be dispersed throughout the entire volume of the nucleus, without any particular relationship to the envelope. The envelope consists of the usual double membrane, perforated by nuclear "pores," and ribosomes may be detected on its outer surface.

Consistent with the evident differentiation of the nucleus is the differentiated appearance of the ER. In the nuclear region this membrane system occurs both as a highly-branched, tubular smooth form and as short cisternal elements of rough ER (Fig. 15). However, away from the nucleus (Fig. 16), the reticulum is often discerned as profiles of long cisternae, invariably studded with ribosomes, which, in surface view show distinct aggregations into polyribosomal forms (see also Bonnett and Newcomb 1966). Furthermore, the majority of the ribosomes which occur free in the ground substance are also present as polyribosomal aggregates (Figs. 6, 7, 9, 11, 16, 17, and 21).

Figs. 6 and 7. The outer wall and cytoplasm in *Avena sativa* var. "Rodney." Note the strongly lamellated inner region of the wall, which, at higher magnification (Fig. 7) shows a herring-bone pattern. The cytoplasm has a dense ground substance and some polyribosomes. Fig. 6: ×12,600 Fig. 7: ×59,400.

Figs. 6 and 7.

Another type of cytoplasmic inclusion deserves special mention (Figs. 15, 17, 18, and 22). It consists of a large globule, up to 2 μ in diameter, which may be distinguished readily from a vacuole because it lacks a limiting membrane. These droplets are common both in var. "Ajax" and in var. "Victory," and their osmiophilia mirrors that of the cuticle matrix. Thus, in var. "Ajax" the droplets are pale and the cuticle matrix is much less electron dense than that of var. "Victory," in which these bodies are extremely osmiophilic (compare Figs. 3 and 18, and Figs. 8 and 17). Very large droplets occur in var. "Ajax" (Fig. 17) and it is this variety which shows the extremely large multivesicular elements in the wall (Figs. 9 and 10). It is tempting to suggest that these bodies represent cutin precursor and one might imagine that from time to time this material escapes from the cell and becomes the large multivesicular elements already allustrated. Polyribosomes in the ground substance, and profiles of the ER and mitochondria often seem to be in close proximity to these droplets (Figs. 15 and 17) and the possible significance of these associations is brought out in the discussion.

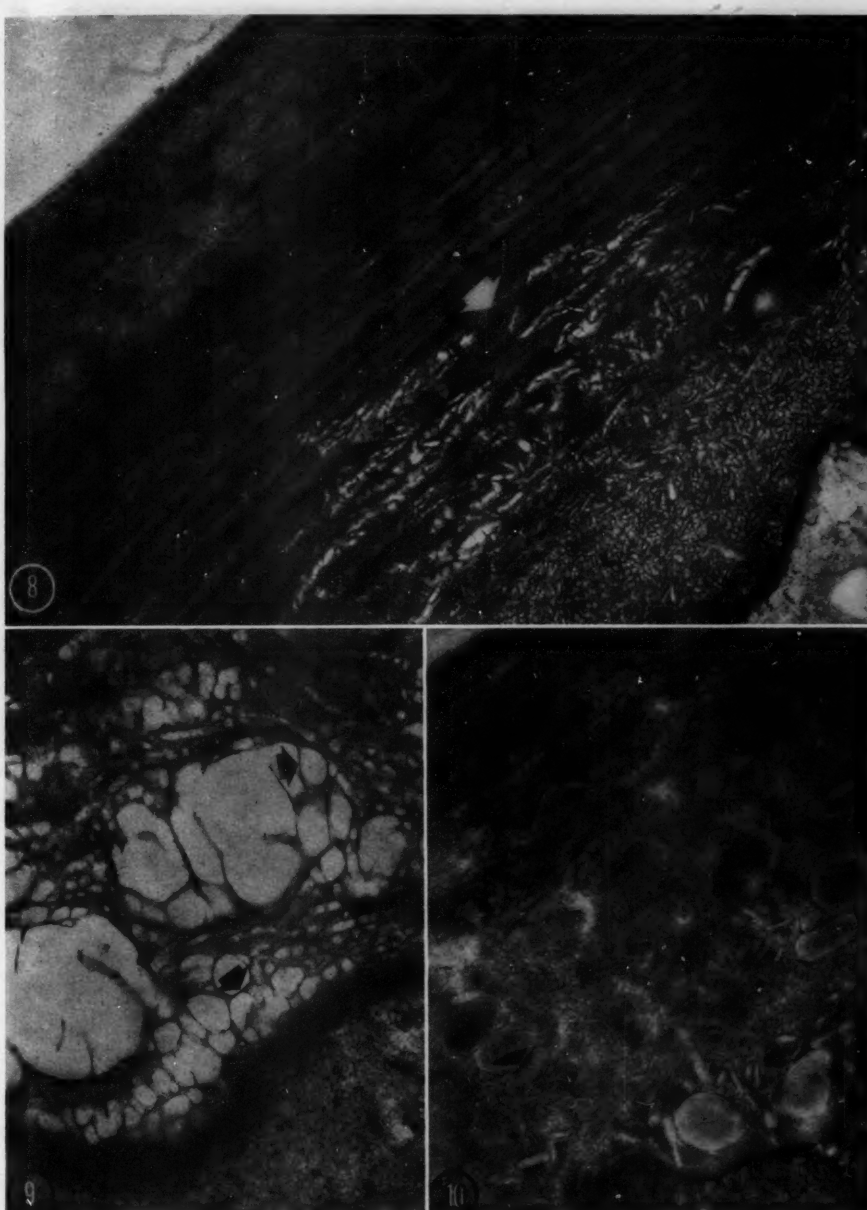
In oats, these cells usually show one large central vacuole, but the ring of peripheral cytoplasm also contains numerous smaller vacuoles (Fig. 15). Usually a well-defined tonoplast limits these vacuoles but it has been our experience that this membrane system of the cell is the most difficult to preserve intact. Often, large areas of the cytoplasm appear to be in direct contact with the vacuole but in such cases, one can sometimes discern an irregular array of membrane elsewhere in the vacuole, where it clearly does not belong (Fig. 15, asterisk). It is likely that these arrays of displaced membrane are missing remnants of the tonoplast which have become dissociated from the cytoplasm at some stage in the preparative procedures. The vacuolar sap of these cells invariably contains fibrillar material of relatively high electron contrast (Figs. 3, 12, 15, and 21) and on occasion, quite large inclusions have been seen (Fig. 20). These larger bodies are visible in the light microscope (O'Brien and Thimann 1965) under phase contrast, but nothing is known of the chemical composition of any of this material in the vacuoles.

The cytoplasm/cell-wall interface shows several interesting features. Microtubules (Ledbetter and Porter 1963; 1964) have not been observed in these cells at any time. However, this may not mean that they are absent *in vivo* because we have been unable to fix these cells satisfactorily with glutaraldehyde at room temperature. It appears (L. G. Tilney, personal communication) that microtubules are not always preserved in cells

Figs. 8–10. The outer wall and cytoplasm in *Avena sativa* var. "Ajax."

Fig. 8. The inner layer of the wall is distorted by multivesicular elements, although the lamellation continues undisturbed in areas in which they are absent (arrow). ×21,000.

Figs. 9 and 10. Two different forms of the multivesicular elements. In Fig. 9, the elements appear empty, and are not surrounded by any membrane, although the wall material at their margins does have a slightly enhanced electron density (arrows). ×42,000. In Fig. 10, the elements appear to contain some material whose electron contrast closely resembles that of the cuticle matrix (see Fig. 8). ×20,600.



Figs. 8–10.

fixed in glutaraldehyde at 0° C. If these tissues are fixed in acrolein/OsO_4 at room temperature, the overall preservation of the cytoplasm is excellent but the electron density of the ground substance is so great that it would mask the microtubules even if they were preserved. The density of the ground substance is less when acrolein fixation is carried out at 0°C, but again microtubules have not been seen.

However, in material fixed in glutaraldehyde/OsO_4 at 0° C, there are several features to note. The cytoplasm, is, of course, bounded by a clear-cut, triple-layered membrane, but this membrane is *not* applied smoothly and uniformly to the wall (Figs. 15, 16, and 19); instead the wall surface is irregularly roughened and the plasmalemma follows a tortuous path. Where the membrane is withrawn from the wall (perhaps by slight plasmolysis) one may see extensive areas of the cell cortex which seem to lie outside it (Fig. 19). In such cases, a dictyosome is often present in the nearby cytoplasm. A more striking arrangement sometimes occurs beneath the outer wall. Quite large invaginations of the plasmalemma occur which enclose within them material whose fine structure resembles that of the wall nearby (Fig. 21). This resemblance is maintained even when different fixatives are used. Thus, both the inner layer of the wall and the content of these invaginations consist of an array of coarse granules in material fixed solely in OsO_4 (Fig. 22). However, in material fixed with glutaraldehyde/OsO_4, this granulated appearance of the inner layer of the wall is replaced by a finely-fibrillar texture and the invaginations also show only fibrillar material (Fig. 21). With this fixation, these invaginations are commonly associated with an amorphous, dense body which lies just outside the plasmalemma (Fig. 21, arrows). These structures have been observed most frequently beneath the outer wall, and their significance is sharply enhanced by the fact that they form a conspicuous organelle beneath the epidermal walls of growing cells.

C. The Stomata

The fine structure of the cuticle and middle layer of the outer wall of the guard cell is remarkably similar to that of the other cells of the epidermis (Figs. 23 and 24). The cell wall of the guard cells is covered with a thick layer of cuticle which extends into the upper part of the pore and forms a raised peg (Fig. 24). However, the cuticle is much thinner over the inner surface of the guard cells (Fig. 25). The fine structure of the cuticle

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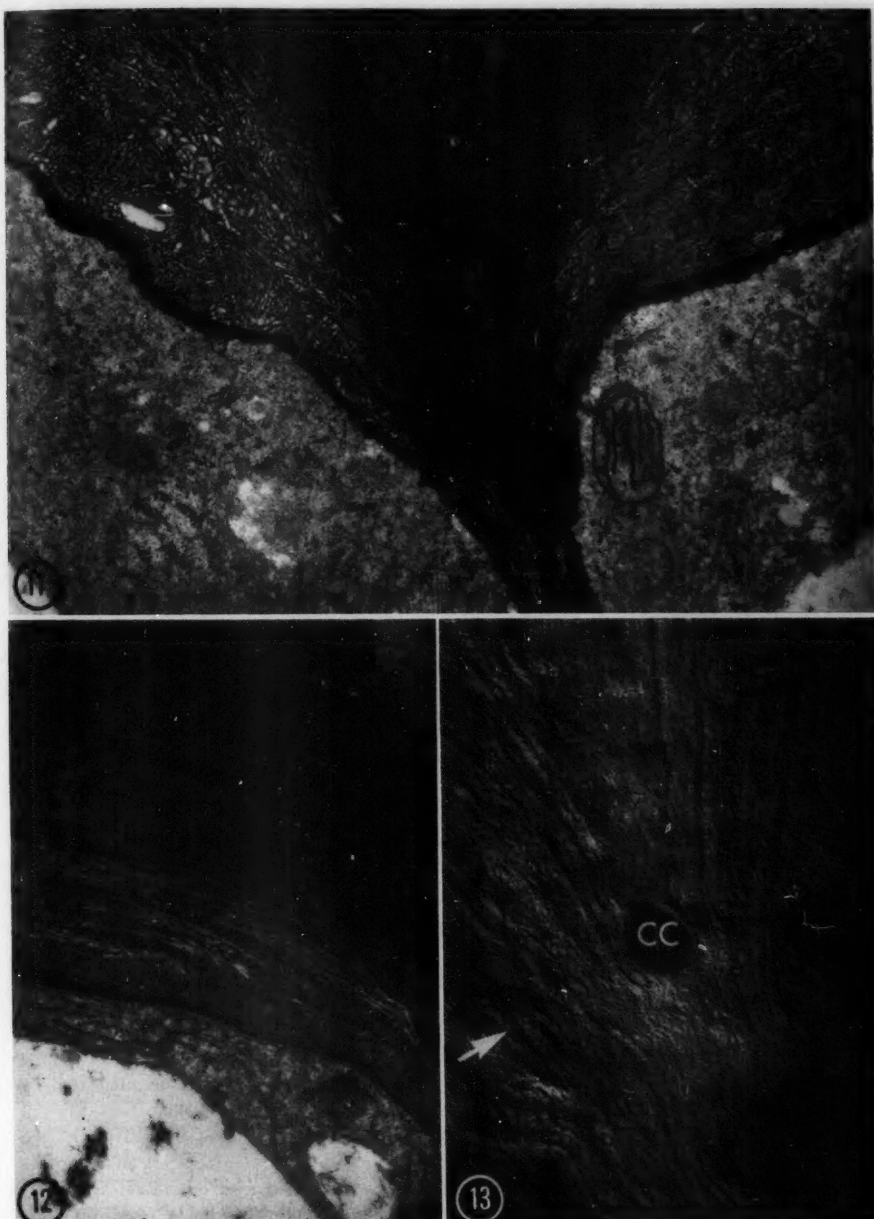
Fig. 11. The corner thickening and cytoplasm (TS) in *Avena sativa* var. "Ajax." Note the multivesicular elements in the thickenings. ×19,000.

Fig. 12. Part of the corner thickening (TS) in *Avena sativa* var. "Victory." Note the smaller multivesicular elements of this variety and the lamellations of the inner wall. ×20,600.

Fig. 13. A shadowed transverse section of the corner thickening of var. "Victory;" the shadow direction is marked by the arrow. Note the lamellae in the inner layer of the wall, the absence of lamellae in the middle layer, and the cutin cystolith. ×29,000.



Figs. 11–13.

is similar to that already described and cutin cystoliths even occur at some depth in the middle layer of the wall. Again, the structure of the middle layer closely approximates that of the other epidermal cells. However, the inner layer of the wall shows finely granular material with a prononced electron density which is attributable to the presence of lignin.

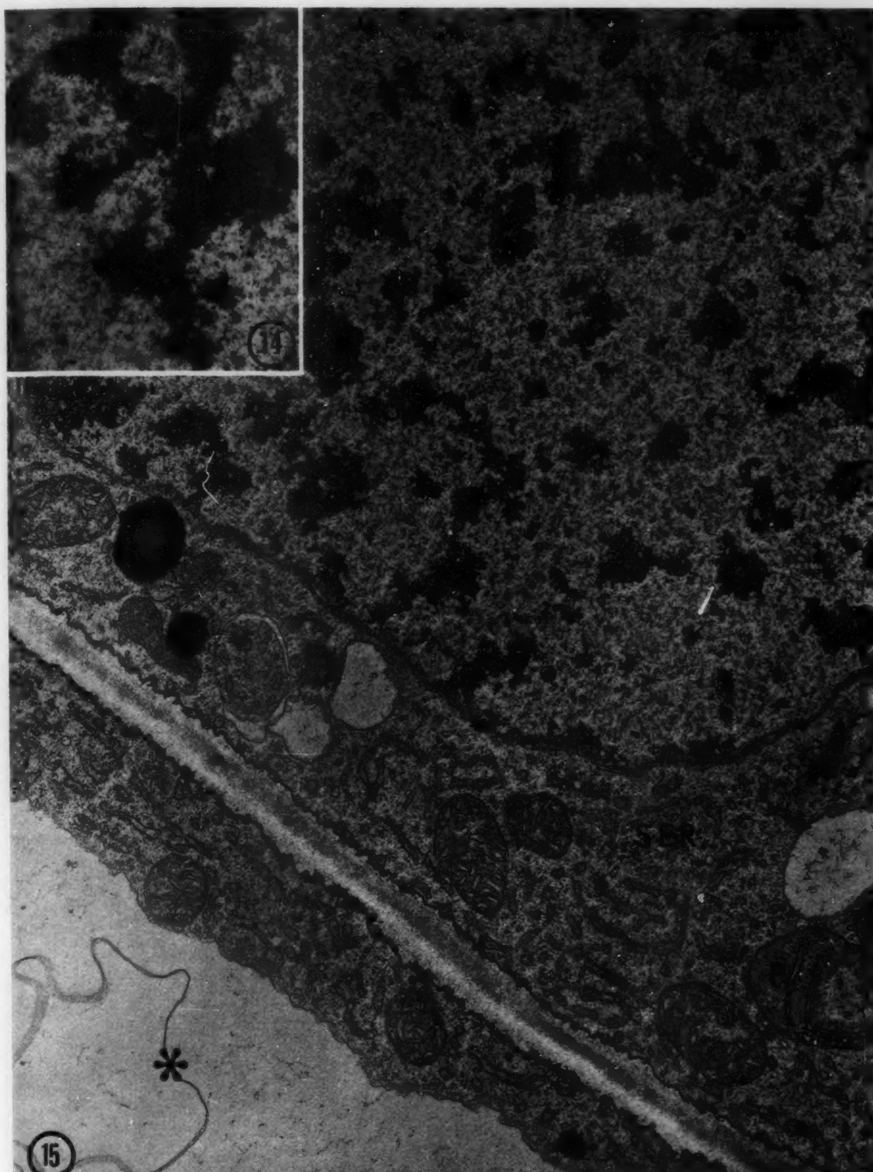
The chromatin of the nuclei of the guard cells is even more densely aggregated than is that of the other nuclei of the epidermis (Fig. 25). In addition to this evident differentiation of the nucleus, the cytoplasm is also markedly differentiated. Dictyosomes and mitochondria are numerous and the ground substance (rich in polyribosomes) is traversed by extensive cisternae of rough ER (Figs. 23–25). In contrast to the plastids in the guard cells of leaves (Brown and Johnson 1962), the plastids of these guard cells are the only ones in cells of the epidermis which do turn green in the light. Furthermore these cells are unique in the coleoptile because they combine the capacity for greening with the storage of very large grains of carbohydrate (O'Brien and Thimann 1965, and Fig. 26). Normally, the parenchyma cells which can green in the light have smaller carbohydrate grains than those which cannot green. In the EM, the plastids show a reasonably well-developed lamellar structure, and a prolamellar body (Fig. 26). Since those observed here were all from plants which had received unknown amounts of red light, (but no white light), it is not possible to say what the final structure of these plastids would be if grown in the light.

Finally, the guard cells are the only cells of the epidermis in which we have detected microtubules. These structures are present in good numbers in the cortex, especially beneath the differentially-thickened wall (Fig. 26). This observation confirms the observations of Ledbetter and Kawakami (unpublished) on the guard cells of *Phleum* leaves; similar arrangements of microtubules have also been seen in the differentiating guard cells of *Phaseolus vulgaris* (O'Brien, unpublished).

Discussion

A. The Cell-wall

Unfortunately, these observations are not readily related to existing knowledge of wall structure because the structure of the cells at the apex of the coleoptile has never been presented. Indeed, apart from the work of Perry (1932), which has already been discussed (O'Brien and Thimann 1965), other investigators mention this region only in passing (e. g., Avery and Burkholder 1936; Roth 1956; Bonnet 1961). On other types of epidermal cell a vast amount of information has been gathered (see Linsbauer 1930 for the early references and Esau 1965, Ch. VII for an excellent summary of later work). The structure and composition of the epidermal cell-wall has also received a good deal of attention in those xerophytic species which have differentially-thickened and cutinized walls similar to those studied here. In their recent book, Frey-Wyssling and Mühlethaler (1965) have summarized much of the available in-



Figs. 14 and 15. The nucleus and perinuclear cytoplasm in var. "Victory." Displaced tonoplast at asterisk. Fig. 14: ×20,600; Fig. 15: ×21,700.

formation on these walls, and their Fig. J-27 b presents a detailed interpretation of the outer epidermal wall in the leaf of *Clivia*. This diagram shows the inner layer to be rich in cellulose and lamellated and to grade into a region designated as the primary wall, external to which is a layer rich in pectins. The rest of the wall is interpreted as an adcrustation, the cuticle, which consists of a cuticular layer and an outer layer of cutin.

With some important modifications, the outer epidermal wall of the coleoptile can also be interpreted in this way. The inner layer is certainly lamellated (Figs. 3, 6 and 7) and it is rich in cellulose (Bayley et al. 1957). It is not clear whether one should regard this layer as a secondary wall in these cells of the coleoptile tip because we do not know when the layers are deposited. The outer layer, *ol*, (which is rich in hemicellulose and poorer in cellulose) is continuous with the primary wall of the radial walls (Figs. 1–3) and is analogous to the region designated as the primary wall of *Clivia*. The cuticular layer of *Clivia* corresponds closely to the region designated as cuticle in Fig. 1, and it seems reasonable to suggest that the pectin lamella in *Clivia* corresponds (at least in part) to the layer, *pl*, of Fig. 1. If there is a layer of cutin *sensu stricto* over these coleoptile cells it might correspond either to that very thin region on the surface of the cuticle which is free of the fibrillar material (see Fig. 4) or perhaps to the electron-transparent layer which always seems to separate the microorganisms (which commonly adhere to the surface) from the first visible layer of cuticle.

However, the epidermal walls of the coleoptile differ from those of *Clivia* in one major point that has far-reaching implications. Frey-Wyssling and Mühlethaler (1965) show the pectin lamella of the outer wall of *Clivia* as a layer which is continuous with the middle lamella region of the radial (anticlinal) walls. Studies on acrolein fixed tissue strongly suggest that the fibrillar reticulum of Figs. 4 and 5 which corresponds in part to the layer *pl* of Fig. 1, is richer in polyuronides than the rest of the wall. Quite clearly, this layer does not occur in the radial walls; instead, it forms a continuous layer across the top of the outer layer of the wall. Furthermore, we have already seen that the cutin cystoliths are surrounded by similar material (Fig. 5), even when they occur at the base of the radial walls (Fig. 1). These facts raise several points which bear both on the concept of middle lamella in general and upon the nature of epidermal cell-walls in particular.

It is becoming clear that the cell plate (which becomes the middle lamella) is formed by the fusion of vesicles derived from the dictyosomes (Whaley and Mollenhauer 1963). In addition, Whaley and his associates have demonstrated repeatedly that vesicular material, again attributable to the dictyosomes, is incorporated not only into the mucilagi-

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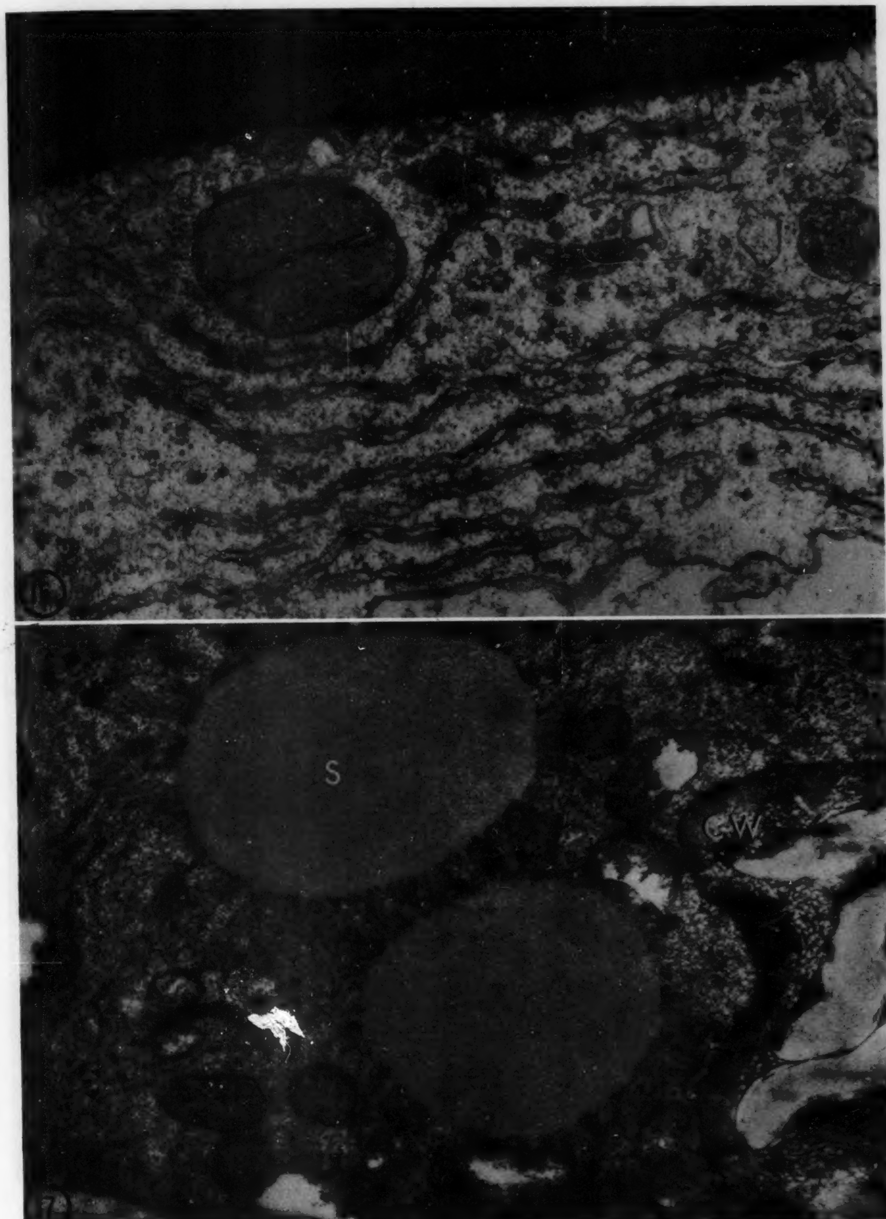
Fig. 16. The rough ER in the cytoplasm of var. "Garry," at a distance from the nucleus. ×40,000.

Fig. 17. Spherosomes in the cytoplasm of var. "Ajax." Note also the large multivesicular elements in the wall nearby. ×29,000.



Figs. 16 and 17.

nous walls of the root cap, but into the outer epidermal wall of the root (see e.g., Mollenhauer et al. 1961; Leech et al. 1963). This last is most important because the *outer* walls of epidermal cells are not formed by the addition of material to a cell-plate. In wheat, pulse-chase experiments and EM autoradiography of root-cap cells labelled with tritiated glucose (Northcote and Pickett-Heaps 1966) have shown that the dictyosomes became labelled first and that subsequently this label was transferred to the wall. As these authors point out, it is unlikely that the label in the dictyosome would survive the preparative procedures for EM unless it was in material of relatively high molecular weight. Chemical analysis showed that most of the label was recoverable after hydrolyses as galactose, and the authors point out that it is probably in pectic substances. This paper clearly constitutes the first clear-cut evidence for the incorporation of a polysaccharide into the wall. When the labelled pulse was followed by a sufficiently long time (about 1 hr.) in unlabelled sugar, the label disappeared from the dictyosomes and became localized in the wall, often at some distance from the cytoplasm. It would appear, therefore, that this type of polysaccharide may migrate through the fibrillar matrix of the wall.

The observations presented here suggest that similar phenomena may be occurring in the epidermal walls of the coleoptile. Dictyosomes are present in good numbers in these cells and there is certainly evidence for the incorporation of wall material (Figs. 21 and 22). One should remember that in this study, the cells are from coleoptiles 25–35 mm long and these cells have obviously synthesized a great deal of wall already; perhaps the period of greatest activity of the dictyosomes is over. The appearance of the outer wall in Fig. 1 does bear a superficial but striking resemblance to a paper chromatogram; perhaps the polyuronide-rich layer which entirely covers the surface of these cells represents the accumulation of the more water-soluble pectic substances, which have moved through the fibrillar frame-work of the wall. It was mentioned earlier that it is difficult to resolve any layer in the radial walls which resembles the middle lamella of parenchyma cells. It was shown many years ago that the anticlinal walls of epidermal cells act as pathways for the loss of water from leaves by cuticular transpiration (Wylie 1943). Perhaps the polyuronide-rich layer of these anticlinal walls has been swept to the surface and is united with the same material which has moved through the outer wall.

Several other publications deal with the fine structure of the cuticle and outer epidermal cell-walls (see, e.g., Bolliger 1959; Frey-Wyssling and Mühlethaler 1965; Scott et al. 1958). Unfortunately, the methods of specimen preparation used did not preserve a level of detail

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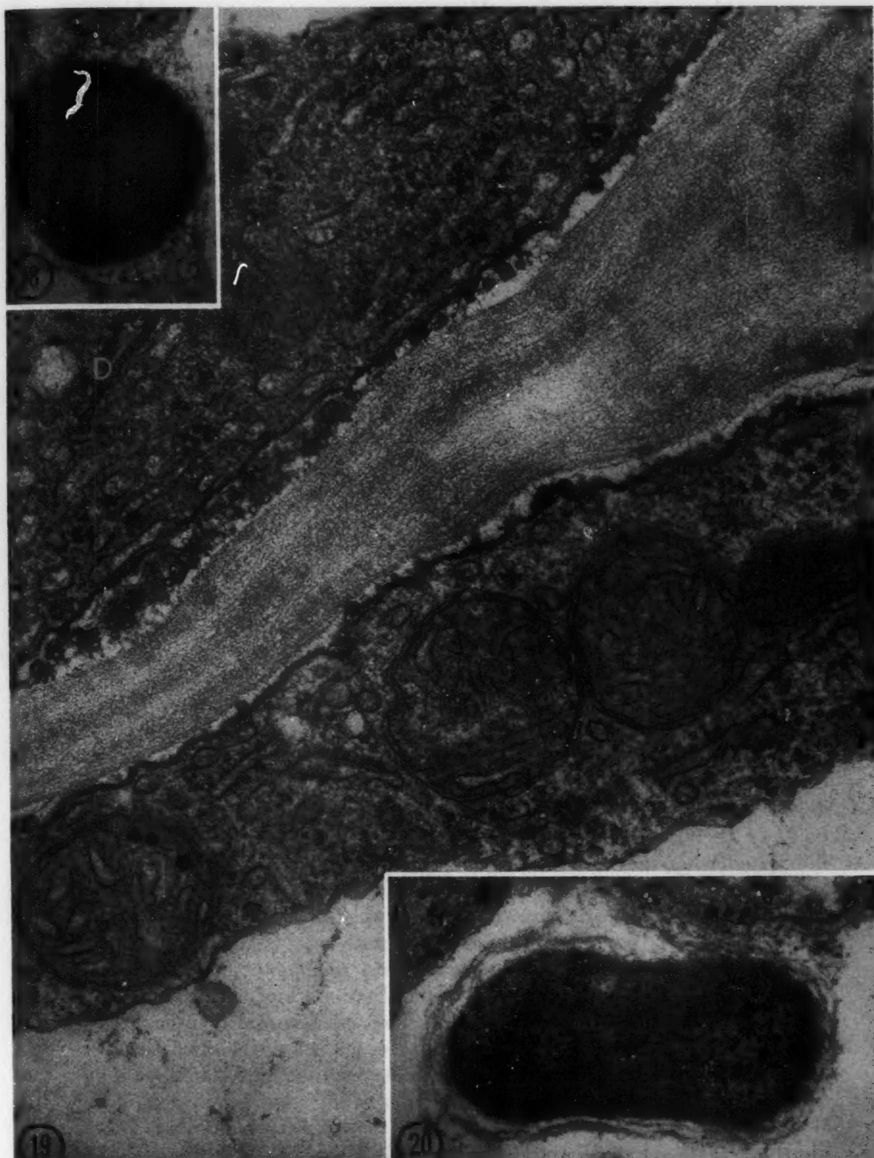
Fig. 18. A spherosome from the cytoplasm of var. "Victory." ×46,000.

Fig. 19. Cytoplasm and radial wall of var. "Victory." The plasmalemma appears to have "cut off" a layer of the cortical cytoplasm. Note the dense granules in the mitochondria. ×71,300.

Fig. 20. A vacuolar inclusion in var. "Victory." ×54,000.



Figs. 18–20.

comparable to that shown here. However, a recent study of several species (Bayer et al., personal communication) has shown that one of the major types of epidermal wall structure is that shown here in coleoptile cells. Furthermore, the observations agree with ours (and with those of many of the authors above, especially Frey-Wyssling and Mühlethaler (1959) that the matrix of the cuticular layer is osmiophilic. Roelofsen (1952) has atributed the behavior of this layer in polarized light to oriented platelets of wax. However, leaf waxes (in contrast to the lipids of seeds) do not contain any appreciable concentration of unsaturated lipids (Eglinton and Hamilton 1963) but are rich in long-chain, paraffinic esters, which one would not expect to react with OsO_4. Furthermore, Frey-Wyssling and Mühlethaler (1965) claim that pure cutin contains no double bonds, and this is certainly supported by their micrograph of the cutin of *Clivia,* which is not osmiophilic. One is forced to conclude that the matrix of the cuticular layer is made up in part of unsaturated lipids which lose their osmiophilia as they mature into cutin, presumably by oxidation or hydrogenation of their double bonds.

The lamellation of the inner layers of the wall supports a view which is gaining acceptance, namely that cellulose deposition in cells which are not rapidly expanding does not take place at a uniform rate or with a constant orientation of the microfibrils (see Wardrop 1964). The 12–18 lamellae observed do not correlate with any reasonable periodic stimulus to which the cells could have been exposed. However, since it is not known how much of this wall had been synthesized before germination, judgement on the significance of the lamellation must be reserved.

It remains to attempt an explanation of the multivesicular elements (MVE) of the wall. Eglinton and Hamilton (1963) state that "the wax originates in the epidermal cells as oily droplets that reach the surface of the plant via minute canals (plasmodesmata) penetrating the thickened cell-wall-the cuticular layer." Unfortunately, there is very little solid evidence to support this belief, though Frey-Wyssling and Mühlethaler (1959), in a study of cuticle formation in *Echeveria* leaves have illustrated what they believe to be droplets of "procutin" in transit to the leaf surface. It is difficult to see just how a large droplet of lipid up to 2 μ in diameter could migrate through a frame work of cellulose, hemicellulose and pectins as an intact droplet. No doubt much smaller lipid droplets (but still perhaps greater than 500 Å in diameter) might escape from the cell and come to lie outside the plasmalemma. Suppose now that the presence of such a droplet of lipid at the cell surface inhibits wall synthesis (perhaps by inhibiting cellulose synthesis or hemicellulose incorporation). In that case, the MVE would represent regions of inhibited wall-synthesis due to such a mehanism and one would attribute the variation in size of the MVE, and even their presence or absence, to variations in the size of the lipid aggregate which escaped from the cell. It seems to be more than a coincidence that the very large MVE in var. "Ajax" occur in the same variety that has very large lipid droplets in the cytoplasm. Furthermore the time required for the included material to escape from these large

droplets would depend upon several variables and this could eplain the cell-to-cell variation in the content of these vesicles. The large lipid droplets in the cytoplasm are discussed below.

Finally, the cutin cystoliths pose a question. These bodies do occur at some depth in the outer wall and are surrounded *on all sides* by the same fibrillar reticulum which occurs at the junction of the cuticular layer. They

Figs. 21 and 22. Large "vesicles" which may represent stages in the incorporation of material into the wall.

Fig. 21. Glutaraldehyde/OsO_4. The content of the vesicles is fibrillar and a dense body is present close to the point of origin of the invagination of the plasmalemma. $\times$32,000.

Fig. 22. A similar structure seen in OsO_4 fixation. The content of the invaginations ist granular as is the inner layer of the wall. Contrast the preservation of the cytoplasm in the two fixations. $\times$18,000.

have exactly the same structure even when they occur at the base of the radial walls. It is not easy to understand why these structures should form in the first place, and it is evident that we are still unaware of all of the forces which are at work in shaping the structure of these epidermal walls.

B. The Cytoplasm

A major difficulty in interpreting the fine structure of higher plant cells is the lack of available information on the biochemical significance of some of the organelles. The activities of the mitochondria (see Lehninger

1964), plastids and probably of the dictyosomes (see Northcote and Pickett-Heaps 1966) are moderately well understood, but there is very little unequivocal evidence for the biochemical significance of any of the single membrane systems of the higher plant cell. There is no clear idea of the activity of the smooth and rough ER, the nuclear envelope, the tonoplast and the plasmalemma. Indeed, it is not certain that polyribosomes make protein in plant cells, though the recent observations of Bonnett and Newcomb (1966) are certainly consistent with this idea. These deficiencies in our knowledge force one to rely heavily upon analogy and intuition in the interpretation of the fine structure of plant-cell cytoplasm.

However, the tip cells of the outer epidermis display several features which are worthy of comment. First, there is good evidence that material is being incorporated into the wall, for both membranous and fibrillar material is seen outside the plasmalemma (Figs. 19 and 21). If the situation in these cells is similar to that described by Northcote and Pickett-Heaps (1966), one might expect that the dictyosomes, which are numerous, would be more proliferated. But these cells are from the apex of a mature coleoptile and it is likely that the major period of activity of wall synthesis is over. Furthermore, there is no evidence that the dictyosomes synthesize cellulosic polysaccharides, and the inner layers of these walls are richer in cellulose and poorer in non-cellulosic polysaccharides. It will be of interest to investigate similar cells in the apex of very young coleoptiles.

Mitochondria are numerous and plastids are poorly developed, and since these cells normally grow in the dark, it is perhaps not surprising that the number and structure of these organelles should reflect the heterotrophic conditions of growth which prevail. However, although some ferns, aquatics, and a few terrestrial higher plants do have chloroplasts in the epidermis of the leaves, by far the majority of terrestrial plants lack chlorophyll in the epidermis, even in cells which are illuminated. Perhaps this proplastid condition in epidermal cells, as seen in the coleoptile, is just another example of this widespread phenomenon.

Figs. 23–26. The guard cells of var. "Exeter."

Fig. 23. The outer wall and pore of the stoma. Note the cuticular peg and the bacteria which partly occlude the pore. ×9,000.

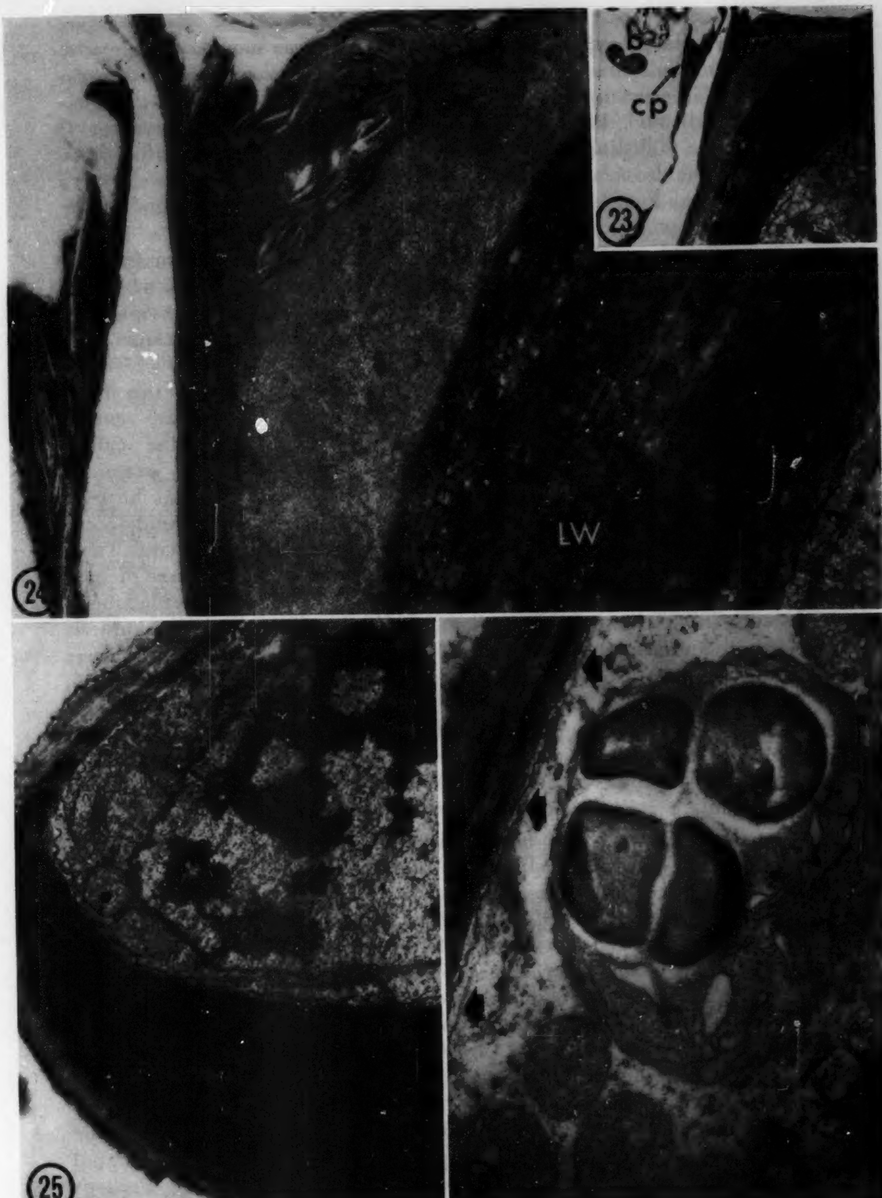
Fig. 24. A detailed view of the section seen in Fig. 23. The cuticle and outer layers of the wall are closely similar in structure to the corresponding layers of other epidermal cells, but the lignified layer is much more electron dense. ×48,000.

Fig. 25. The bottom wall of the guard cell. Note the dense aggregates of nuclear chromatin. ×19,500.

Fig. 26. The plastid of the guard cell, with its large grains of carbohydrate, and small prolamellar body. Note the microtubules beneath the wall (arrows). ×30,000.

All micrographs are from material fixed in glutaraldehyde/OsO_4 at 0° C, except for Fig. 22, which was fixed in phosphate-buffered 2% OsO_4.

Abbreviations to legends: *B* = bacteria; *C* = cuticle; *CC* = cutin cystolith; *CP* = cuticular peg; *CW* = cell wall; *D* = dictyosome; *IL* = inner layer of the wall: *LW* = lignified wall; *OL* = outer layer of the wall; *P* = plastid; *PL* = polyuronide layer; *S* = spherosome; *SER* = smooth endoplasmic reticulum.



Figs. 23–26.

The marked invaginations of the surface membrane of these cells are suggestive of some type of pinocytotic activity. This might correlate with their heterotrophic nutrition. Alternatively, infoldings might arise because membrane is constantly being added to the surface (as wall material is incorporated), and the perimeter of these cells is constantly shrinking. It is impossible to distinguish between these possibilities with the information available, but the occurrence of similar irregularities of the surface in very rapidly expanding cells (see Cronshaw 1965) does argue against the latter suggestion.

Two other organelles, the large lipid droplets and the mitochondria, call for special mention. Scott et al. (1958) demonstrated droplets, presumed to be cutin precursors, in epidermal cells of onion, and it is reasonable to equate these droplets with the highly refractive bodies (which stain with a great many lipid stains) called spherosomes (Frey-Wyssling and Mühlethaler 1965). Thus, it is suggested that the large droplets whose osmiophilia mirrors the osmiophilia of the cuticle in the Avena varieties studied, are the precursors of the cuticle and would be recognized as spherosomes in these cells if examined by phase contrast microscopy. Stumpf (1965) suggests that at least some of the enzymes which are necessary to synthesize such lipids occur in the microsome fraction, while others occur in the soluble proteins. These lipid droplets do show a morphological association with cisternae of both the rough and smooth ER, and polyribosomes frequently occur in the ground substance in close association with them. Furthermore, since the synthesis of these materials requires considerable amounts of ATP, it is of some interest that mitochondria are often associated with these bodies (Figs. 15, 17). In some instances, lipid synthesis in animal cells is associated with the smooth ER of the cells (see, e.g., Parakkal and Matoltsy 1964; Senior 1964 and references cited therein) and as Fig. 15 shows, the perinuclear area of these epidermal cells does contain a significant amount of such a tubular membrane system.

One feature of the mitochondria is worth mentioning. Figs. 15, 17, 19 show that they diplay a wealth of the so-called dense granules, which may be sites at which calcium salts are deposited within the mitochondria (Lehninger 1964; Peachey 1964). Both of these workers have been able to modify the amount and morphology of these deposits by suitable alteration in the mineral metabolism of the mitochondria. Althrough André and Marinozzi (1965) have expressed doubts about the exact nature of these deposits, their morphology is correlated with the degree of ion accumulation by the mitochondria. Deposits of inorganic salts are extremely common in the walls and cytoplasm of epidermal cells (Esau 1965). Presumably, this salt arrives in these cells through that part of the transpiration stream which is lost as cuticular transpiration. Indeed, there is reason to believe that in many leaves, the epidermal walls constitute a preferential pathway for water movement from the leaf (see Wylie 1943). Perhaps the mitochondria of coleoptile cells regulate their ionic environment and this activity may be reflected in the abundance of dense granules.

In a recent paper, Lowary and Avers (1965) have refocused attention upon an obvious and well-documented fact: in higher plants, the structure of the nucleus is closely correlated with the degree of differentiation of the cell and with its physiological activity. As examples, one may cite the alterations in nuclear structure of the tentacle cells of the insectivore, *Drosera*, which accompany the feeding of protein to these cells (Konopka and Ziegenspeck 1929); the different and exact sequences through which the nuclei of cambial initials in pine pass as they differentiate into xylem or phloem cells (Bailey 1920); the different nuclear cytology of the products of many asymmetric cell divisions, such as those which form two-celled pollen grains and those which produce many of the epidermal structures of leaves (Bünning 1957). Modern techniques and concepts (e. g., see Karasaki 1965; Littau et al. 1964) will have to be brought to bear upon these systems before we can hope to interpret the more subtle changes which accompany the less dramatic differentiations of parenchyma cells.

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Observations on the Fine Structure of the Oat Coleoptile

II. The Parenchyma Cells of the Apex

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With 24 Figures

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Introduction

In previous papers (O'Brien and Thimann 1965; Thimann and O'Brien 1965) the histology of the coleoptile apex has been described and illustrated in some detail. It was shown that the apex consists of three tissues: epidermis (inner and outer), vascular system, and parenchyma. The cells of the parenchyma range between two extremes. On the one hand, the cells nearest the outer epidermis on the phloem side of the vascular bundles contain plastids which turn quite green in the light. On the other hand, the parenchyma of the avascular tip (which closes the coleoptile) and the cells nearer the inner epidermis fail to develop any appreciable green pigment in the light. The rest of the parenchyma cells lie between these two extremes, and it is clear that in the shaft of the coleoptile the capacity to turn green shows a marked radial gradient towards the outside.

Although parenchyma cells constitute the bulk of the living tissues of higher plants, surprisingly little data has been published on their fine structure. Such information as there is comes largely from cells of the coleoptile. Thus, Arrigoni and Rossi (1962; 1964) make brief mention of the parenchyma cells but their work is concerned primarily with the vascular system. Wardrop and Foster (1964) have studied the parenchyma and epidermis of young coleoptiles in material fixed in permanganate, while Thornton and Thimann (1964) and Cronshaw (1964) have both reported on the crystal-containing bodies of these cells in the oat coleoptile. The walls of this tissue have been studied more extensively

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(e.g., Mühlethaler 1950; Setterfield and Bayley 1958; 1959; Böhmer 1958; Wardrop 1956; Wardrop and Cronshaw 1958) and Roelofsen (1965) has recently clarified and integrated most of the available information.

In this paper, we are concerned with the fine structure of the parenchyma as it occurs in the uppermost 1–4 mm of a 25–35 mm oat coleoptile; this essentially eliminates any consideration of the rapidly growing cells. In this paper, our interest in the fine structure of these cells is three-fold. First, we are concerned with the difference(s) in structure which accompany the variation in pattern of greening. Secondly, it is almost certain that it is these uppermost cells which synthesize auxin, transport it polarly to the growing zone, perceive phototropic stimuli and react to these stimuli by the induction of net lateral transport of auxin. The perception of geotropic stimuli is not limited to the apical cells but takes place to at least 10 mm below (Dolk 1930). Thirdly, these cells are representatives of a large class, namely, parenchyma cells which have ceased division and slowed down in growth. As such, their structure has signifiance which may outweigh their importance to an understanding of the coleoptile.

Materials and Methods

Most of the observations which follow are based upon 1–4 mm tips taken from coleoptiles (25–35 mm long) of *Avena sativa* L., var. "Victory." The observations have been confirmed on several other varieties of *Avena sativa* ("Garry," "Exeter," "Ajax," and "Rodney") and upon *Avena fatua* L. The conditions of growth of the coleoptiles and the methods of specimen preparation for electron microscopy (*EM*) have been described in detail (O'Brien and Thimann 1965; O'Brien 1967). In brief, the tissues were fixed overnight in 1.5–3% glutaraldehyde, washed, postfixed in 2% OsO_4, dehydrated in acetone and embedded in Araldite.

Observations

A. General

In the light microscope (O'Brien and Thimann 1965), the parenchyma of the apex is seen to consist of thin-walled, relatively large cells (30–50 μ in greatest dimension), which have a large central vacuole. The nucleus seems to lie always against the wall, embedded in the thin parietal layer of cytoplasm which surrounds the vacuole. The cytoplasm stains weakly with acidic dyes (in contrast to the marked acidophilia of the epidermal cells) but is moderately basophilic and in favorable sections one may resolve mitochondria and stained profiles of what appears to be the endoplasmic reticulum (ER). If the coleoptile tips are fixed in the vertical position, all of the plastids which are packed with carbohydrate are seen to lie against the bottom wall. This behavior of the plastids has for a long time been correlated with the geotropic sensitivity of the coleoptile (see the review by Audus 1962), but recently, Pickard and Thimann (1966) have cast doubt on the relationship and have shown that geotropic curvature can occur, albeit somewhat more slowly than usual, in the total

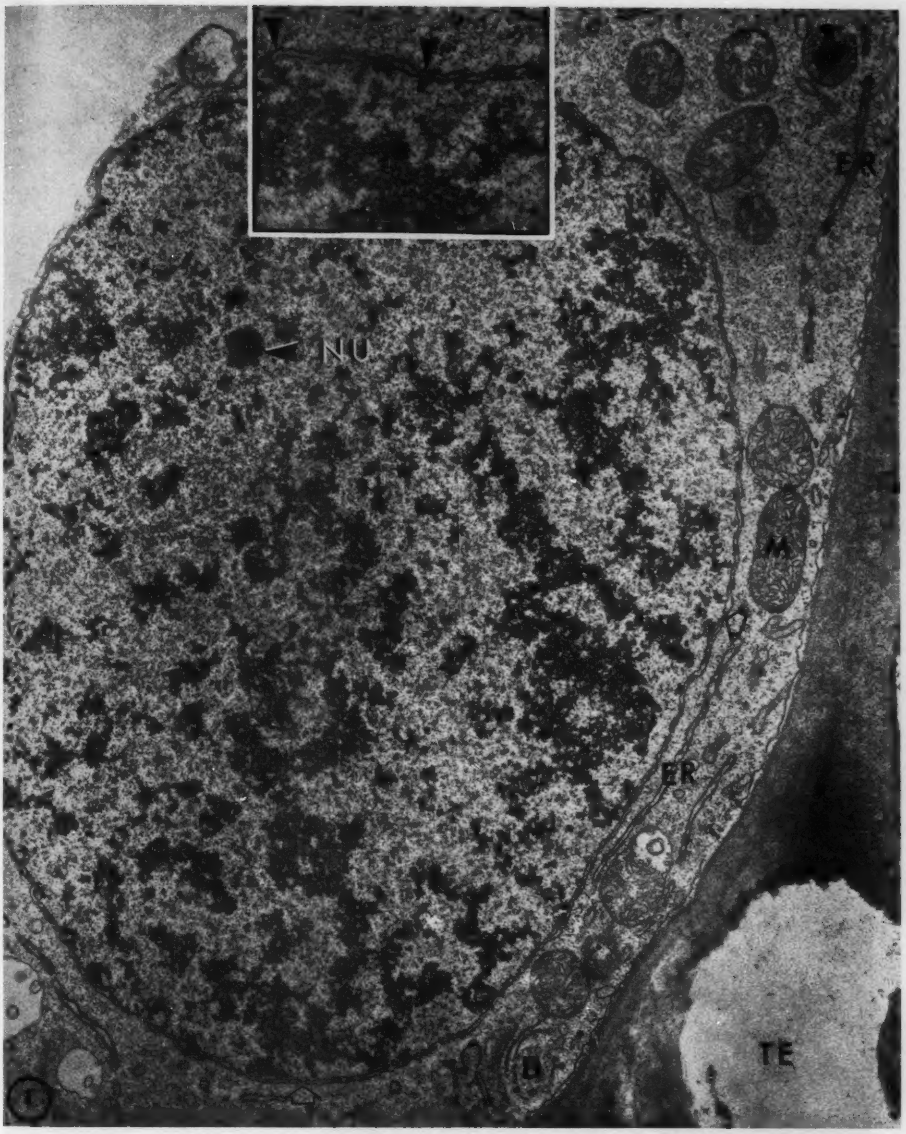


Fig. 1. A general view of the nuclear region in a parenchyma cell of the tip of *Avena sativa*. The cell is near the inner epidermis and borders a tracheary element (*TE*) which is part of the cap which terminates the bundles. Note the nuclear pores (open arrows), paucity of ribosomes on the nuclear envelopes, small nucleolus (*NU*). ×18,900.

Inset: Detail of nuclear envelope (the unit membranes are resolved at the arrows) and chromatin. ×46,000.

absence of carbohydrate-rich plastids. The degree to which the plastids stain with acidic and basic dyes is correlated, as mentioned above, with the position of the cell in the coleoptile and is related to the degree to which the plastids turn green in the light.

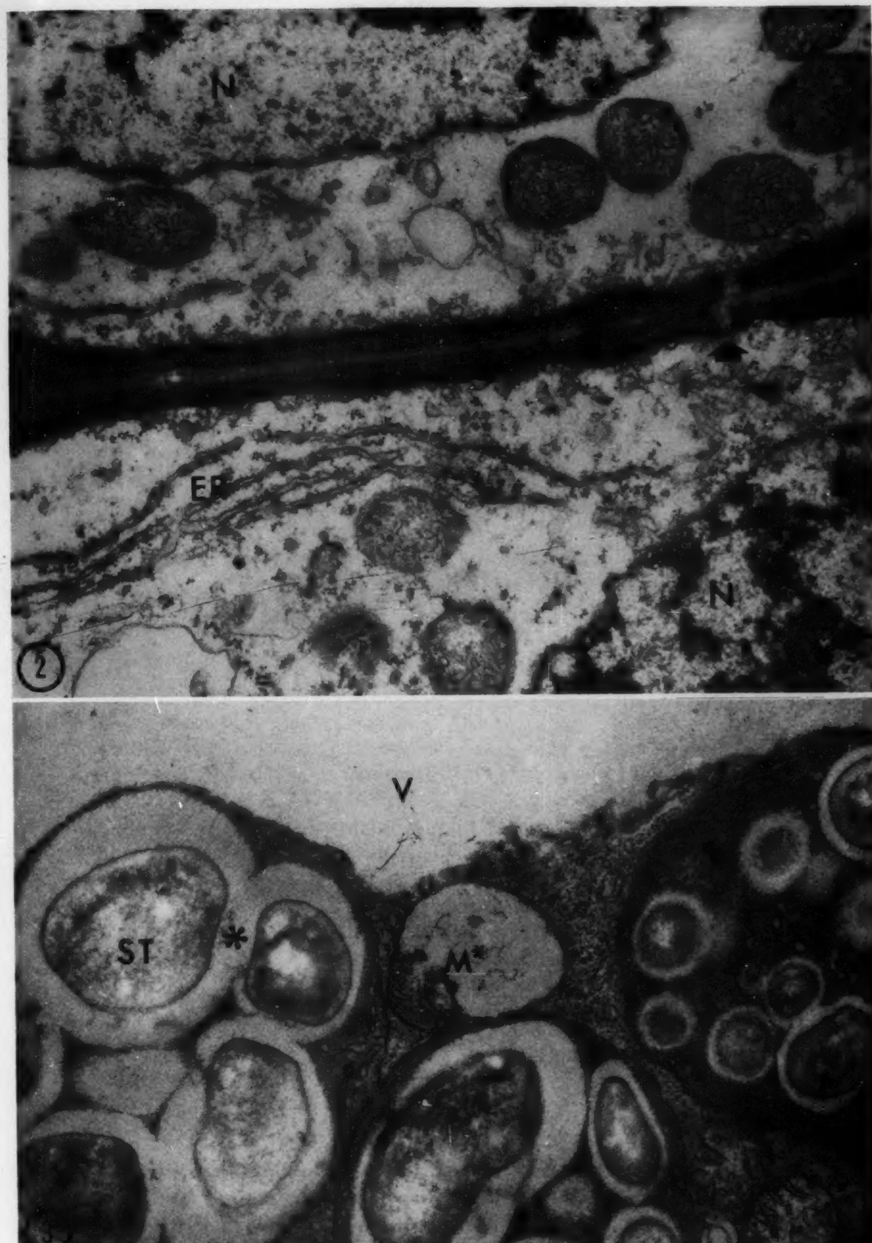
The fine structure of the nucleus and the general appearance of the cytoplasm in these mature parenchyma cells is certainly consistent with the suggestion that such cells are differentiated (Figs. 1 and 2). Furthermore, their fine structure does not suggest that they are particularly active in a metabolic sense. The nucleus consists of an array of chromatin aggregates reminiscent of the structure seen in epidermal cells (O'Brien 1967), but these aggregates seem to be somewhat more dispersed than was the case in the epidermal cells. Again, the chromatin does not seem to be arranged with any obvious relationship to the nuclear envelope, which consists of the usual double membrane (Fig. 1, inset) perforated by nuclear pores (Fig. 1, open arrows). In contrast to the nuclear envelopes in some cells of the vascular system, the outer membrane of the nuclear envelope here is not invested with ribosomes. Mitochondria are reasonably numerous and their numbers and structure are about what one might expect of cells which are totally heterotrophic (Figs. 1 and 2). Dictyosomes occur sporadically throughout the cytoplasm but show no evidence of any major activity, a fact again consistent with the slowly growing state of these cells (Figs. 1, 3 and 16).

The apparent quiescence of these cells only serves to emphasize the structure of the rough ER (Figs. 1 and 2). In the region of the nucleus, the ER is sparse and in sections is represented usually by a few profiles of tubular elements. However, in the region away from the nucleus, and especially parallel to the side walls, one may discern profiles of a very extensive cisternal form. Sometimes only a single cisterna is evident, but more commonly the sheets are arranged as stacks of 2–4 (Fig. 2; up to 8 have been seen). In surface view, the cisternae appear to be studded with ribosomes, arranged in polyribosomal aggregates. In addition, in occasional sections some of these cisternae are seen to enclose some type of granular product (Fig. 13). Smooth ER is present in these parenchyma cells but apparently is much less evident than in the lipid-rich epidermal cells. Most commonly, it is associated in the parenchyma cells with the crystal-containing bodies (see below).

The most striking difference between the fine structure of the epidermal cells and that of the parenchyma cells concerns the ground substance. In the parenchyma cells it is very much less electron dense than was the

Fig. 2. LS of the lateral walls of two parenchyma cells in the avascular tip. The arrow marks the position of a plasmodesma. ×27,300.

Fig. 3. Plastids of parenchyma cells of the avascular tip. The section passes through these plastids in a region which is rich in carbohydrate, and free of the stromacenter (see Fig. 4). The mitochondrion labelled with an asterisk has been damaged during specimen preparation, and the space* between the stroma of the plastids and the grains of carbohydrate (*ST*) is probably due to shrinkage of the grains. ×31,900.



Figs. 2 and 3.

case in the epidermal cells: indeed, it often appears as if it had been diluted or extracted (Figs. 2, 4, 12 and 17). In addition, the free ribosomes (which are present usually as polyribosomal aggregates) do not "stain" as strongly as their counterparts in the epidermal cells.

The extracted appearance of the ground substance and the relatively weak staining of the ribosomes is very much less apparent in those parenchyma cells whose plastids can green in the light (cf. Figs. 5, 6, and 16 with Figs. 4 and 17). It would appear that these cytoplasmic characters vary with the position of the cell in the organ in the same way that the plastid characteristics vary. They are not simply due to differences in fixation. This possibility was considered seriously because the cytoplasm of the parenchyma cells of the avascular tip and of those cells which lie near the inner epidermis are often poorly preserved. However, the difference in appearance between the epidermal cells and the two extremes of the parenchyma cells is maintained in tissues fixed solely in OsO_4 or in glutaraldehyde. In addition, later experiments (O'Brien and Hall, unpublished) have confirmed that the difference is also visible when these tissues are fixed in several different mixtures or sequences involving acrolein and glutaraldehyde prefixation, and post-fixation with OsO_4. It seems unlikely, therefore, that this difference can be dismissed as a simple fixation artefact, and an alternative explanation is offered in the discussion.

B. The Plastids

The plastids of the parenchyma cells of the coleoptile are large (up to 10 μ in diameter) and range in structure between the extremes illustrated in Figs. 3 and 4, and Figs. 5 and 6. The fine structure of those plastids which will green in the light is similar in all respects to that described already in detail by Gunning (1965 a, 1965 b; Figs. 5 and 6). They contain one or more prolamellar bodies, a large stromacenter, and a sparse array of lamellae; both the prolamellar body and the stromacenter contain numerous osmiophilic granules. The plastids also contain regions which resemble bacterial nucleoids, and the stroma is rich in particles which resemble the chloroplast ribosomes (see also Jacobsen et al. 1963). In addition, these plastids contain grains of carbohydrate which are usually absent from the leaf tissue. These grains stain with the procedures of specimen preparation used here and are always surrounded by an electron-transparent area, which is interpreted as a shrinkage artefact. In short, there is little obvious difference between these plastids in the coleoptile and those of the primary leaf.

The plastids which will not green up in the light (Figs. 3 and 4) are similar in some respects to those which will green. Thus, they contain regions which resemble bacterial nucleoids, have a well-developed stromacenter, and a few quite long lamellae. However, they differ in two major respects: there is no indication that these plastids contain a prolamellar body or that they have chloroplast ribosomes. In view of Gunning's work (1965 b) on the structure of the prolamellar body in oat plastids, it is very likely that these two phenomena are related (see the discussion

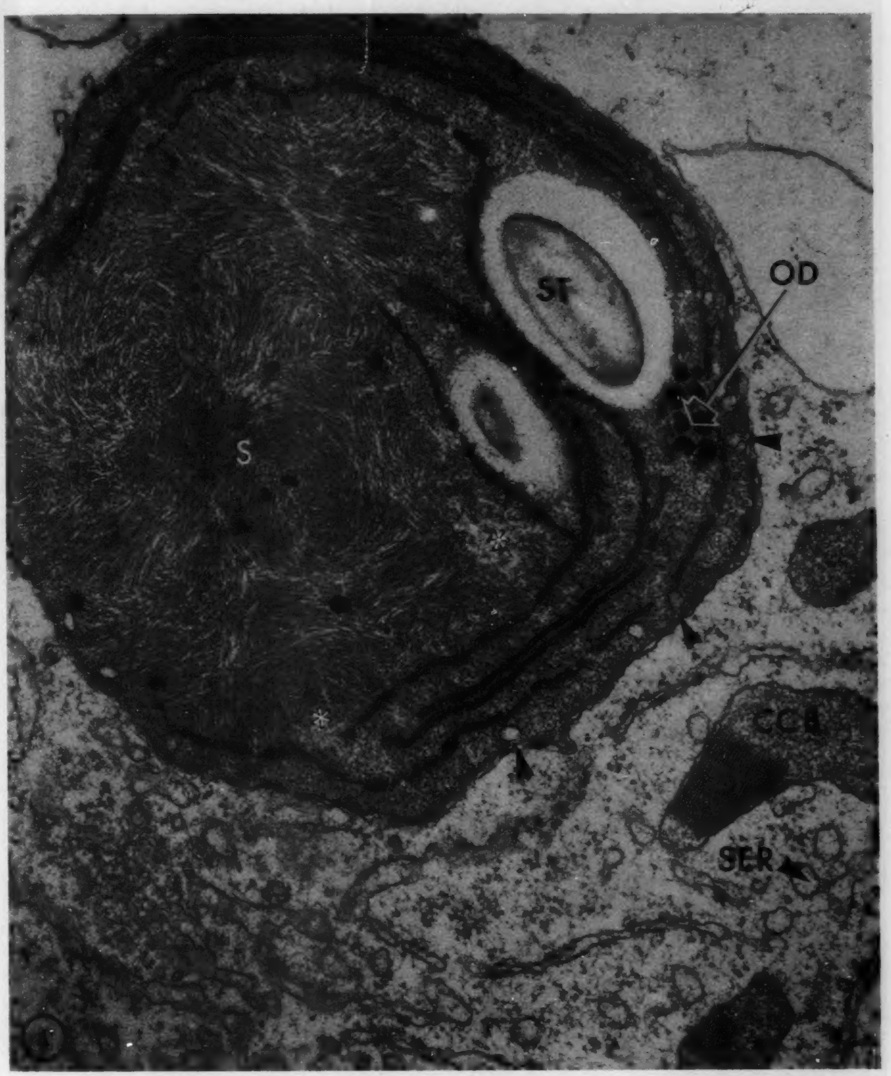


Fig. 4. A plastid from the avascular tip, sectioned in the region of the stromacenter (*S*). Note the nucleoid regions (asterisks), osmiophilic droplets, absence of a prolamellar body, and the blebs of membrane (arrows) which may be sites of pinocytotic activity (see text). ×42,800.

below). In addition, these plastids are much richer in grains of carbohydrate than those which green in the light, and this is supported by the observations with the light microscope (O'Brien and Thimann 1965). Finally, the osmiophilic droplets in these nongreening plastids are traversed by a curious region of low electron density, a phenomenon which has not been observed in the plastids which can turn green.

C. The Plasmodesmata

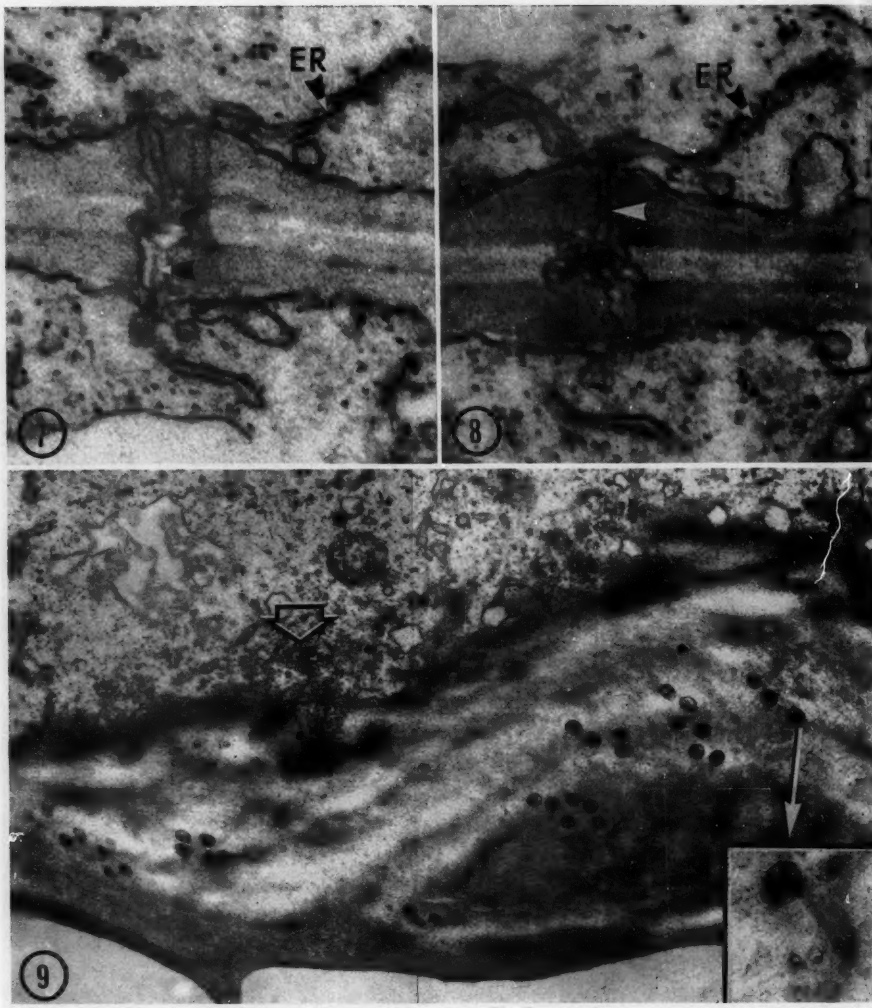
The parenchyma cells of the coleoptile tip transport auxin polarly from apex to base and they respond to a phototropic or geotropic stimulus by the induction of a net lateral transport of auxin (see Gillespie and Thimann 1963; Pickard and Thimann 1964). These facts stimulated us to examine the plasmodesmata quite closely. It has been known for some time that pits occur in all walls of the parenchyma (Mühlethaler 1950) and their structure and behavior during growth has been used as evidence that the walls elongate over their whole surface (Böhmer 1958; Wardrop and Cronshaw 1958). In sectioned material the plasmodesmata are usually cut in various oblique planes but occasionally one sees what appears to be an almost perfectly longitudinal section (LS). The sections illustrated in Figs. 7 and 8 are adjacent to one another (probably 700–850 Å apart), and show several features of interest.

It is clear that the wall is perforated by two canals which lie at slightly different levels. These canals meet in the compound middle lamella region of the wall in an irregular "cavity:" this cavity, and the walls of the pores which traverse the wall, are lined by a membrane which appears to be similar to the plasmalemma (Figs. 11 and 12). Where the pores emerge onto the cytoplasm, the wall is raised up into a tiny mound (reminiscent of a volcano) the neck of which appears to be entirely closed by a mass of electron-dense material (Figs. 7, 8, 11 and 12). Just below the neck, the pore seems to widen out and in the center is a strand of material whose electron contrast is similar to that of the closing plug (Figs. 7, 8, and 11). These strands can be traced into the central cavity but it is hard to decide whether or not they are continuous across it. The central cavity also contains sections of material which appear to be membranous as well as some irregular deposits of electron dense material (Fig. 9, inset, and Fig. 12).

This structure seen in LS allows one to interpret the various appearances which plasmodesmata present in oblique transverse sections (Figs. 9 and 12). In such sections, the regions near the cell surface appear as circular profiles, lined by a membrane and with a central dark core (solid arrows, Fig. 12). In the middle of the wall one intersects the central cavities which again appear as membrane-lined pores (though of larger dimensions).

Figs. 5 and 6. The chloroplasts of the oat coleoptile. Note the prolamellar bodies (*PB*), stromacenters (*S*), and definite chloroplast ribosomes (*CR*). Note also that the density of the ground substance of the cytoplasm of these cells is much higher than that seen in Fig. 4. Fig. 5: ×31,700. Fig. 6: ×44,000.

Figs. 5 and 6.



Figs. 7–9.

Figs. 7 and 8. Serial sections through a plasmodesmatal complex in a cross wall. The "pore" of the canal seems to be closed by a plug of dense material which is continuous with an electron dense core (arrows). The 4 pores seem to anastomose in a central "cavity" in the middle of the wall. The endoplasmic reticulum (*ER*) comes up to, and seems to touch, the cores of dense material. See also Figs. 11 and 12. Both ×75,600.

Fig. 9. A composite micrograph of a low angle section of a wall similar to that seen in Figs. 7 and 8. Note that in cross-section, the plasmodesmata appear different when cut close to the cytoplasm or near the central cavity. The clear arrow shows an aggregation of filamentous material in the cell cortex near the pit field (see also Figs. 19 and 20). ×21,400.

In addition to these structural features, the plasmodesmata in these cells show a most striking relationship to the ER. It is often assumed that the ER is continuous from cell to cell through the plasmodesmata (see dis-

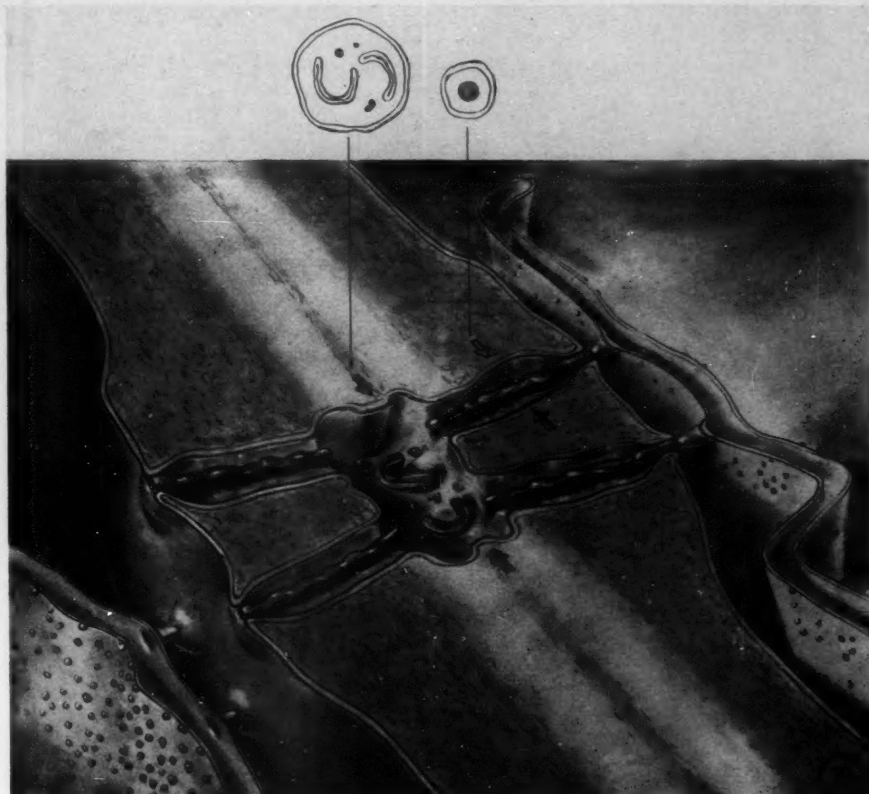


Fig. 10. A diagrammatic reconstruction of the structure of the plasmodesmatal/ER complex, based largely upon the serial sections of Figs. 7 and 8, and upon the appearance of higher resolution micrographs, both in LS and in TS (see Figs. 11 and 12). The ER has been represented as a cisternal element of considerable extent, but it is recognized that in some cases it may be tubular near the plasmodesmata (cf. Figs. 7 and 8 with 14). The appearance which would be seen in TS at two different levels is indicated by the arrows and line drawing. Kindly drawn by Mrs. H. C. Lyman.

cussion); however in these cells the ER comes to and seems to touch the dark plugs of material which close the pores, but the ER does *not* seem to be continuous through them (Figs. 7, 8, and 11). The dark strand which occupies the center of the pore bears no resemblance to a membrane, but

looks more like a spindle fiber; in favorable sections one may even detect some sub-structure within it (Fig. 12, solid arrows).

Fig. 10 is an interpretation of the serial sections shown in Figs. 7 and 8, taken in conjunction with the appearance seen in TS (kindly drawn for us by Mrs. H. C. Lyman). Only two "pores" are shown on either side of the wall but it is to be understood that these are part of a larger number which would constitute the normal primary pit field. The ER is shown in the regions of these "pores" as a cisternal element of considerable extent, since this appears to be the case in many instances (see Fig. 14). However it is likely that in many cases the ER near the plasmodesmata is actually arranged in a tubular form. In either case, a small out-pocketing of it seems to touch each plasmodesmatal plug.

This association between the ER and plasmodesmata is most commonly observed on the cross-walls, i. e., on the walls at right angles to the direction of growth. Indeed, the cisternae of the ER often seem to by-pass the plasmodesmata of the lateral walls (Fig. 2, arrow). If this observation, on further investigation, should prove to be a general one in this organ, it would raise several interesting possibilities, some of which are treated in the discussion.

D. The Crystal-Containing Bodies (CCB)

Since these structures were first described in the coleoptile by Thornton and Thimann (1964), a considerable amount of extra information has been gathered about them. This may be summarized as follows:

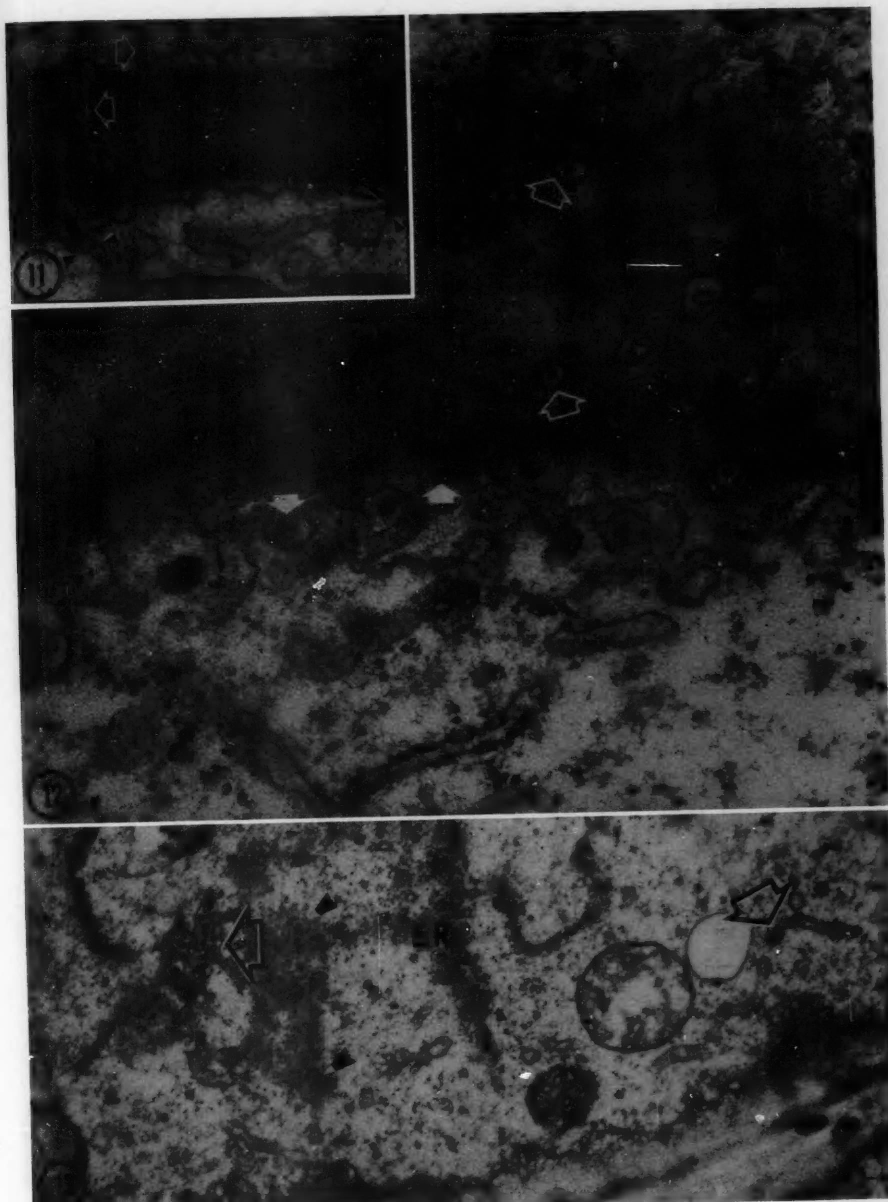
(i) Identical structures occur in all varieties of *Avena sativa* so far examined (var. "Ajax," "Garry," "Rodney," "Exeter" and "Victory") and in *Avena fatua* (Figs. 4, 16–18). These seeds were supplied from widely different locations (Idaho, Saskatchewan, Svaløf) and this lessens the possibility that this structure could be related to a virus infection.

(ii) Similar structures which lack a crystalloid have been found in the coleoptiles of maize and rye (Figs. 23 and 24).

(iii) It was possible to establish that these structures could be seen in the light microscope (Figs. 21 and 22). By correlating the light and electron microscopy, it was found that the CCB occur in the parenchyma cells of the coleoptile at all levels and that they are more numerous in those cells whose plastids lack the capacity to green in the light. They are present in the inner epidermis in good numbers but are seen very rarely in the outer epidermis.

Figs. 11 and 12. Higher resolution micrographs of an LS (Fig. 11) and a TS (Fig. 12) of the plasmodesmatal/ER complex. The membrane which lines the pores is particularly evident (open-arrows) and some substructure can be detected within the core of material which closes the canal (solid arrows, Fig. 12). The association with the ER is particularly clear (Fig. 11, small arrows). Fig. 11: ×34,000. Fig. 12: ×68,300.

Fig. 13. A slightly oblique section of a parenchyma cell which shows the ER in face view. Note the aggregations of polyribosomes (solid arrows), and the accumulation of dense granules within the ER cisternae (open arrows). ×29,800.



Figs. 11–13.

(iv) Their structure does not appear to be different in tissue grown completely in the dark, from that grown in varying red-light regimes.

(v) Identical structures occur in the chlorenchyma of the primary leaf of oats (Gunning 1965 a), but they appear to be less numerous in these cells than in the parenchyma of the coleoptile.

(vi) The structure of these bodies is more pleiomorphic than was realized at first, and it appears that they occur in the cells of the avascular tip both as free bodies and as aggregations. The existence of these aggregations was established in serial sections when it became apparent that what at first appeared to be clumps of individual crystalloids (Figs. 4, 15, 17) were in fact sections of a convoluted tube, with areas of crystallinity separated by varying amounts of non-crystalline material. These tubules of material are often associated with elements of smooth ER, and may perhaps form in association with this phase of the ER. Individual bodies of approximately spherical outline occur as well, however, and may be identified under phase contrast as they stream in the parietal cytoplasm.

(vii) The crystallinity of the crystalloid is not an artefact of OsO_4 fixation, because it can be seen in material fixed only in glutaraldehyde, embedded in Araldite, and stained with uranyl acetate (Fig. 22). This fact also makes it probable that the electron density of the crystalloid is not due to free lipids.

(viii) The CCB may be seen in tissue fixed in acrolein, embedded in glycol methacrylate (O'Brien and Thimann 1965), and stained with acid fuchsin, but they are difficult to distinguish from mitochondria. However in tissue fixed in glutaraldehyde, embedded in Araldite, stained with hot acid fuchsin and examined by phase contrast optics they are sharply differentiated from the mitochondria (Fig. 21) and one may form some estimate of the numbers of CCB per cell. Using these methods we estimate that there are not less than 50 and perhaps up to 200, per cell in the parenchyma of the avascular tip.

(ix) Finally, it is quite evident that the fixed image of the cytoplasm of the parenchyma cells is closely correlated with the state of the membrane which surrounds these bodies. If the membranes of the CCB appear to be intact, the cytoplasm is well-preserved and the ground substance is moderately dense (Figs. 4, 16). This is always the case in the mesophyll cells of the primary leaf (see Gunning 1965 a). However if the mem-

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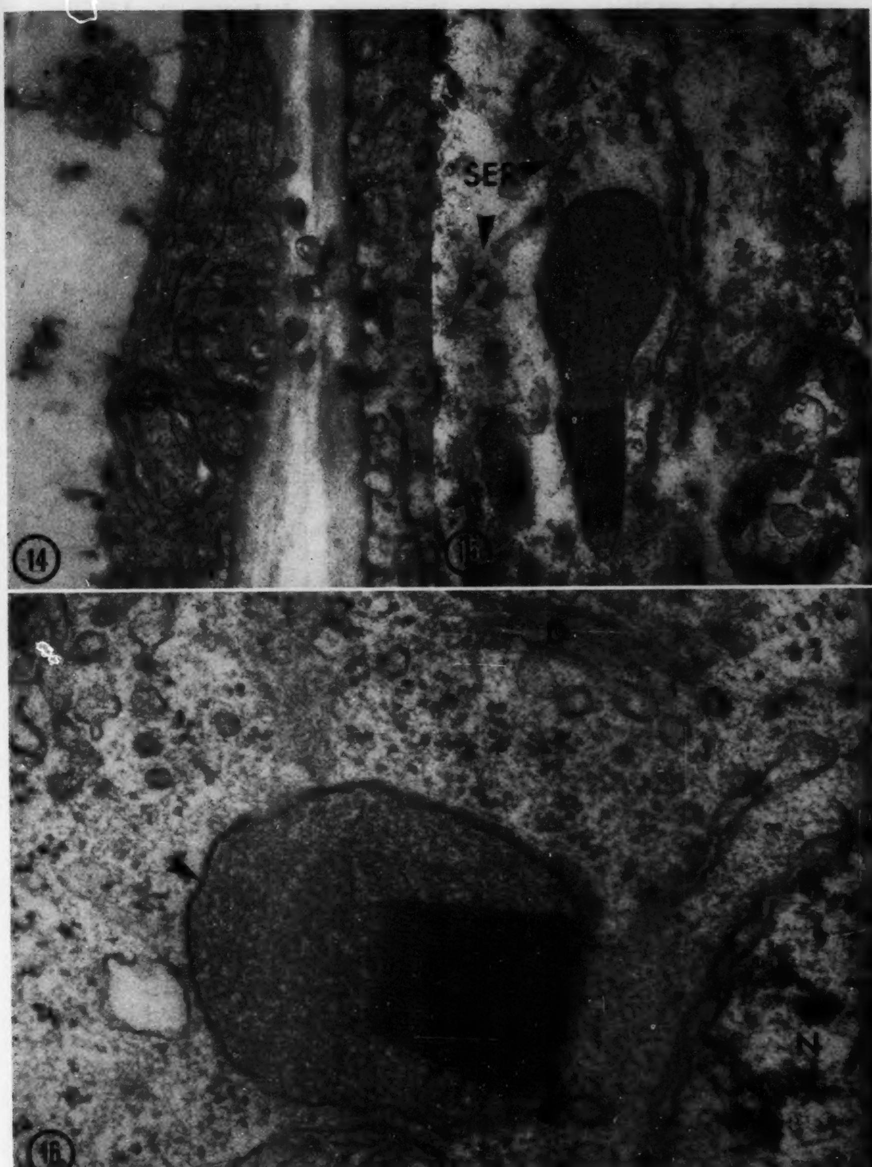
Fig. 14. An oblique view of a pit field which shows that the ER association with the region may have quite a complex form. ×31,700.

Fig. 15. A crystal-containing body (*CCB*) with a well-developed crystalloid, defined membrane, and a considerable amount or organic matrix. This body is part of an aggregation of this material, and note the manner in which the rough ER gives way to smooth ER close to one surface of this structure. ×38,000.

Fig. 16. A CCB from a cell of the coleoptile which contained chloroplasts. The organelle is clearly surrounded by a unit membrane (arrow) and the repeating period of the crystalloid is most obvious (see Thornton and Thimann 1964). ×80,600.



Figs. 14–16.

brane of the CCB appears to be missing or ruptured, the ground substance also appears to be badly extracted (Figs. 17 and 18). These observations support Cronshaw's (1964) suggestion that these bodies may be lysosomes and this point will be taken up in the discussion.

E. Microtubules

Cortical microtubules of the type first described in higher plant cells by Ledbetter and Porter (1963) have not been detected in these parenchyma cells. It is difficult to assess the significance of this observation because Porter (personal communication) has pointed out that microtubules may not be preserved by the fixation used here (glutaraldehyde at 0° C). However, microtubules are preserved by this same fixation in the guard cells (O'Brien 1967) and in the vascular tissues. Thus, it is not clear whether (a) microtubules are absent from the epidermal and parenchymal cells, but present in the guard cells and vascular tissues, or (b) whether they are present in all cell-types, but are differentially preserved with this fixation procedure.

A structure which bears some resemblance to an aggregation of microtubules is commonly present in these parenchyma cells. Usually the filaments are about 220–240 Å wide, and project into the vacuole (Fig. 19). The filaments appear to branch but this may be simply due to overlap of more than one filament within the width of a section. Beneath the aggregates lies a tangled skein of finer filaments which are made evident by the fact that ribosomes seem to be excluded from the area in which they occur. Similar filaments have been detected in the cortex of the cell (Figs. 2, 20) where they often appear to be associated with the region of the pit fields (Fig. 9, open arrow). What relationship these structures have to microtubules, if any, remains to be discovered.

Discussion

A. The Plastids

The variation in plastid structure in the epidermal and parenchymal tissues of the coleoptile is most marked and is clearly related to the ability of the plastid to turn green in the light. Thus, although all the plastids contain areas which resemble bacterial nucleoids (presumably rich in DNA: Swift 1965), and contain at least some lamellae, those which can green in the light invariably contain a prolamellar body and chloroplast ribo-

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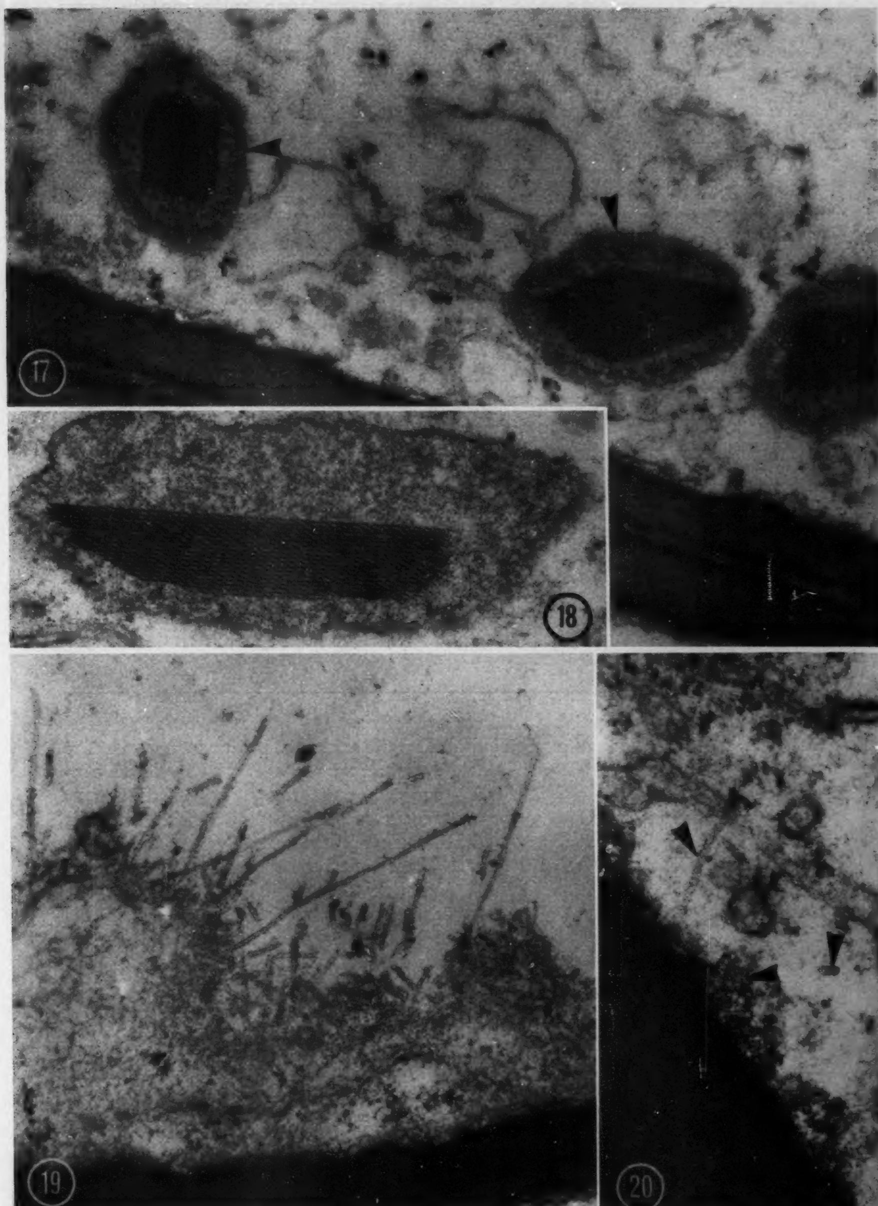
Figs. 17 and 18. CCB in parenchyma cells which lack chloroplasts. Note the apparent absence of a limiting membrane around most regions of the body (arrow: cf. Figs. 15 and 16), and the extracted appearance of the ground substance. Fig. 17: ×34,600. Fig. 18: ×62,000.

Figs. 19 and 20. Aggregates of filamentous structures which bear a superficial resemblance to microtubules. Those in Fig. 19 are projecting into the vacuole, whereas those in Fig. 20 are in the cell cortex (see also the large aggregation in Fig. 9). Fig. 19: ×42,000. Fig. 20: ×65,000.



Figs. 17–20.

somes. In addition, the plastids of the parenchyma cells contain large stromacenters; those of the epidermis (O'Brien 1967) resemble the proplastids of oat roots and lack this structure (Gunning 1965 a).

Gunning (1965 b) has presented an elegant analysis of the structure of the prolamellar body in the plastids of the oat primary leaf, and has suggested that the chloroplast ribosomes may be partly responsible for it. If the ribosomes of the plastid do have such a role, it is easy to understand why prolamellar bodies are absent from plastids which do not appear to contain ribosomes. There might, of course, be a limited number of a special population of ribosomes within the plastid, for in all cases so far examined, there is always some membrane within the plastid which is not part of the prolamellar body, even in plants which have been grown in absolute darkness and whose prolamellar bodies are completely crystalline (Gunning 1965 b; O'Brien and Holowinsky, unpublished).

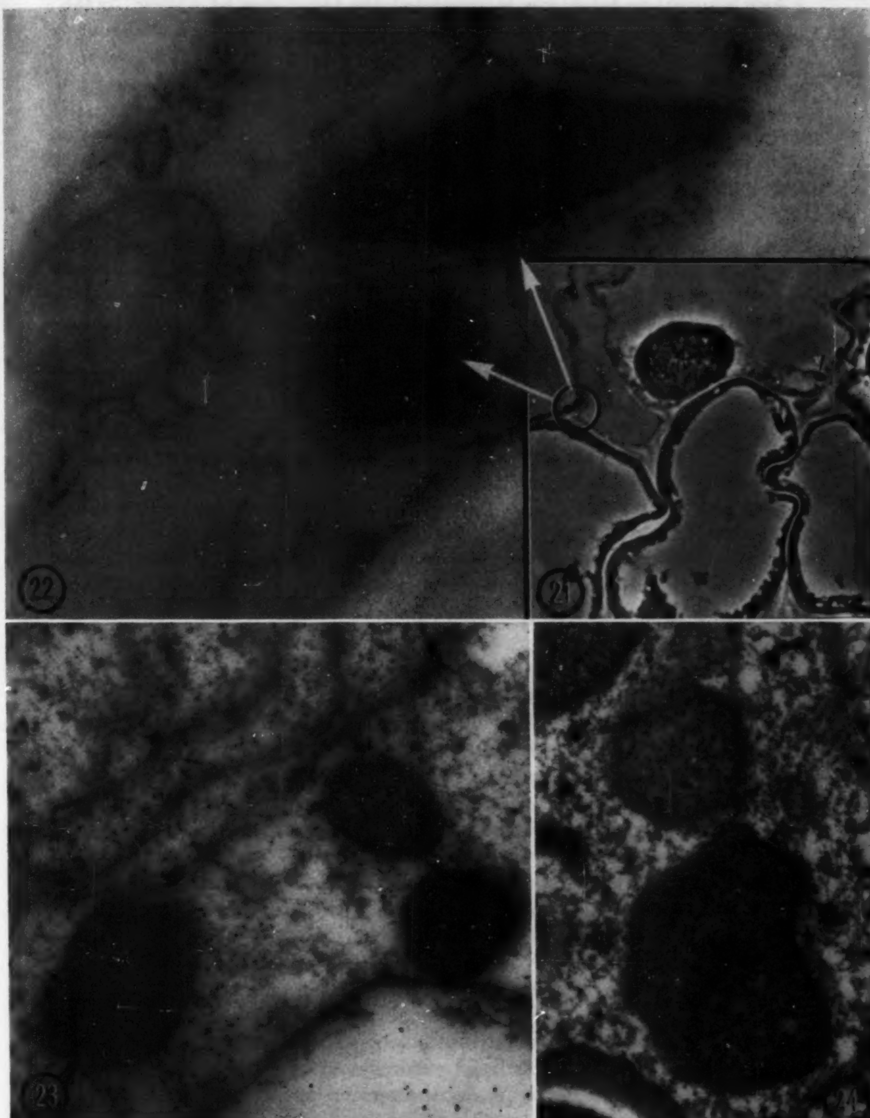
If chloroplast ribosomes are indeed absent from those plastids of the parenchyma which will not green, then one needs to known how these plastids synthesize the massive amount of protein of the stromacenter, and the lipoproteins of the membranes. There are at least three possibilities: (i) ribosomes are present but they don't stain in these preparations, (ii) ribosomes were present at some stage of development of the plastids but have subsequently degenerated, or (iii) the proteins are synthesized outside the plastid and imported into it. Since it is commonly accepted that the membranes of the prolamellar body are aggregates of vesicles which bleb off from the inner membrane of the plastid envelope, these blebs might perhaps reflect a micropinocytotic activity by which proteins are brought into the plastid.

Figs. 21 and 22. Fig. 21 is a light micrograph of an 0.25 μ thick section of material fixed in glutaraldehyde and embedded in Araldite without fixation in osmium. The section was stained with hot (60° C) acid fuchsin, and photographed by phase contrast. The lower cells with folded walls are outer epidermal cells, and numerous dark granules can be seen in the cytoplasm of the nearby parenchyma cells (arrows). The two granules ringed within the circle are seen in an adjacent section in the electron microscope in Fig. 22. These have been stained with uranyl acetate (Watson 1958; O'Brien 1967). Although the contrast is poor, it is quite clear that the granules of Fig. 21 correspond to CCB in Fig. 22. In addition, it is clear that the crystalloid is preserved in the absence of OsO_4 fixation. Fig. 21: ×1,430. Fig. 22: ×60,500.

Fig. 23. Amorphous bodies from the parenchyma of the coleoptile of maize, which may well correspond to the CCB of oats. OsO_4 fixation. ×30,400.

Fig. 24. A membrane-enclosed body from the parenchyma of a rye coleoptile. This body is intermediate in structure between that found in maize and the CCB of oats. ×46,000.

Abbreviations to legends: *CCB* = crystal-containing body; *CR* = chloroplast ribosomes; *D* = dictyosome; *ER* = endoplasmic reticulum; *M* = mitochondria; *N* = nucleus; *NU* = nucleolus; *PB* = prolamellar body; *S* = stromacenter; *SER* = smooth endoplasmic reticulum; *ST* = carbohydrate granules; *TE* = tracheary element; *V* = vacuole.



Figs. 21–24.

Any attempt to relate the structure of the two types of plastid to the general problem of phototropic photoperception is at best highly speculative, but several points may be noted.

First, if the photoreceptor should be a carotenoid, it would most probably be located in a plastid, since carotenoids are not known outside of plastids in higher plants.

Secondly, the experiments showing the distribution of phototropic sensitivity along the length of the coleoptile (see Thimann and Curry 1960 for references) leave no doubt that the tip region is far more sensitive than the base for the high-sensitivity response designated as "first-positive curvature." Strong curvature can be elicited by illuminating only the most apical 0.5 mm (Lange 1927). In the oat coleoptile, the avascular tip (the uppermost 0.25 mm) contains only two cell-types, epidermal cells and parenchyma cells which fail to green in the light (O'Brien and Thimann 1965). Thus the photoreceptor for the first positive response is probably located in these plastids which do not turn green.

Thirdly, all of the work reported here is based upon material which was fixed without maintaining any relationship between the position of the tips on the organ and their position during fixation. Thus nothing can be deduced about the intracellular *distribution* of the organelles in these cells. It is most desirable to compare the fine structure of unstimulated cells with that seen at various times after phototropic stimulation, maintaining orientation of the tissue in the stimulated position while it is being fixed. This is technically difficult, but not impossible.

B. The Crystal-Containing Bodies

Various bodies, usually bounded by a single membrane and containing some form of crystalloid, have been described from a wide range of animal and plant cells. Some of these have already been compared with the CCB by Thornton and Thimann (1964). Some additional types are as follows:

(i) The yolk platelets of certain insect and amphibian eggs (Karasaki 1963 a; 1963 b; Roth and Porter 1964). These bodies are believed to consist of various forms of complex proteins and are regarded as storage proteins. In the oocyte of the mosquito, they seem to be formed by uptake of protein from the blood plasma.

(ii) The granules of eosinophil leucocytes. Under special circumstances, these cells can act as phagocytes and there is excellent evidence (Archer and Hirsch 1963 a; 1963 b) that these bodies contain reserves of hydrolases which are used to attack the ingested material. Thus these organelles may be functionally equivalent to lysosomes which seem to be ubiquitous in animal cells and are believed to be storages of hydrolytic enzymes (see Fawcett 1966; pp. 189–212). Indeed, a special type of these, the uricosome, contains a crystalloid (Afzelius 1965).

(iii) Perner (1965) has recently produced excellent evidence that the aleurone grains in ungerminated pea radicles contain crystalloids. This is suggestive since the aleurone layer of cereal endosperm produces the hydrolases which digest this material (Varner et al. 1965).

(iv) The crystalloids in the sieve elements of certain plants (e. g., Bouck and Cronshaw 1965, pea: Srivastava and O'Brien 1966, pine). In pine and oats, these structures are clearly formed within the plastids.

(v) Finally, miscellaneous crystalloids occur in the vacuole of *Acetabularia* (Tandler 1962), the parenchyma of potato (Marinos 1965) and there are numerous bodies which accompany virus infections (eg., Kallman et al. 1959). Most of these either have no membrane, or have a lattice spacing entirely different from that of the CCB.

The CCB of the oat coleoptile closely resemble the eosinophil granules of the leucocytes. Both have a well-developed crystalloid embedded in an amorphous organic matrix of lower electron density. In addition, there are several apparently disconnected pieces of evidence which suggest the presence of hydrolytic enzymes in the CCB.

First, Tetley and Priestley (1927) have pointed out that as soon as the tip of the coleoptile is ruptured by the primary leaf, those parenchyma cells which lack chloroplasts degenerate. This observation is easy to confirm in any sample of light-grown oat seedlings. It was pointed out above that the CCB are most numerous in the parenchyma cells which fail to green in the light. The observation of Tetley and Priestley is supported by the work of Bonnett (1961) who noted that, in the leaf sheath of oats, the parenchyma cells which lack chloroplasts degenerate and form a lacuna. It will be interesting to determine whether these cells also contain a good number of CCB.

Secondly, there is good reason to expect the occurrence of proteinases in the parenchyma of the young coleoptiles. In ungerminated seeds of wheat and oats, the parenchyma cells are filled with droplets of protein (O'Brien, unpublished), and closely resemble the cells of the scutellum. (The primary leaf tissue, on the other hand, is poor in this material.) Within 30 hours of germination, almost all of this protein has disappeared from the cells of the coleoptile, and is replaced by vacuoles. Thus, the CCB might be reserves of hydrolases which are not used up in this initial proteolysis. It will be remembered that Avery and Linderstrøm-Lang (1940) showed that the cells of the avascular tip (rich in CCB) have a higher content of peptidase per cell than any other region of the coleoptile.

Thirdly, the micrographs presented above show that the state of the cytoplasm tends to be correlated with the state of the membranes of the CCB. The cytoplasm of the parenchyma cells which contain chloroplasts is always better preserved than that in cells which lack them, and the former not only have fewer CCB, but also (as in the cells of primary leaf) their CCB always seem to have an intact membrane. Hence, these latter cells may have undergone postfixation autolysis; indeed, hydrolytic enzymes are notoriously resistent to fixation, especially when the fixative is (as used here) glutaraldehyde at 0° C (see Sabatini et al. 1963). This may in part explain also the difficulty of preserving microtubules in these cells.

Finally, Cronshaw (1964), who has also suggested that these bodies may be lysosomes, has pointed out that they occur in differentiating xylem elements. The complete disappearance of the content of mature tracheary

elements is certainly dramatic and suggests that the cytoplasm is totally hydrolyzed by the release of destructive enzymes. In our own studies of the vascular system of the coleoptile, CCB have also been encountered in completely mature tracheary elements. It may be that the crystalloids which seem so common in the sieve elements serve a similar purpose when the mictoplasm of the functioning element is finally destroyed (see next paper).

It is important to realize that the comparable structure in other species may not have a crystalloid, for this almost certainly depends upon the exact nature of the proteins involved. The same problem has arisen in the case of the uricosome which seems to be the only microbody which has a crystalline core (Afzelius 1965). Indeed, the structures seen in maize and rye coleoptiles certainly lack a regular crystalloid, but are probably related to the CCB of oats. If these bodies are in fact stores of hydrolases, they should be widespread and perhaps some of the "unknown bodies" reported in plant cells are of the same type. Clearly, differentiating xylem, especially that produced by the cambium, should be an excellent place to look for these structures.

C. The Plasmodesmata and the ER

It is particularly in connection with auxin transport that the ER/plasmodesmatal complex presents features of interest. Although the cells were *not* fixed in the same orientation as that which pertained in the intact organ, nevertheless there is reason to believe that the ER tends to be arranged parallel to the lateral walls, which, in turn, is parallel to the direction of auxin transport. In addition, the ER shows a definite relationship to the plasmodesmata in the cross walls and on occasions, the cisternae have been shown to contain granules which can be detected quite close to the plasmodesmata (Fig. 13). It is therefore suggested that the ER may act as a pathway for the intracellular transport of auxin, and that the polarity of transport is in some way associated with the evident connections of the ER to the complex plasmodesmata. To test this suggestion, it will be most interesting to examine the ER/plasmodesmatal complex in cells which have received a phototropic or geotropic stimulus.

While there is no direct support for this speculation, some indirect evidence is at least consistent with it. Porter (1961) has reviewed the evidence that the ER may function as an intra-cellular transport system and since that time, Caro and Palade (1964) have shown that, in the acinar cells of the pancreas, a pulse of tritiated leucine "moves" from the ER to the Golgi apparatus and finally appears in the zymogen granules. Thus, isotopes which appear first at the basal pole of the cells finally appear at the apical pole, a kind of polar transport within the cell. In the coleoptile during the transport of auxin through the tissues, some of it becomes sequestered (Goldsmith and Thimann 1962). This sequestered auxin is recoverable from the tissue by solvent extraction and it has been found that it is then chemically indistinguishable from the form in which it was

supplied (Winter and Thimann 1966). However, it can also be liberated by crystalline proteinases, which strongly suggests that it is bound to a protein, perhaps within a membrane-limited "space." Since the "bound" auxin is not in the vacuole (Winter and Thimann 1966), and since (after no more than 3 hrs.) it can be recovered chemically unchanged (and therefore has probably not been exposed to the oxidase-peroxidase system), it seems possible that it becomes sequestered within the cisternae of the ER, perhaps in that portion of the system which is not engaged in transport.

Plasmodesmata have been studied a good deal in higher plants (e. g., Porter and Machado 1960; Whaley et al. 1960; Kollmann and Schumacher 1962; Falk and Sitte 1963; Dolzmann 1964) and in two recent books, both Wardrop (1965) and Frey-Wyssling and Mühlethaler (1965) have accepted the notion of many investigators that the ER is continuous from cell to cell through the plasmodesmata. There have been earlier objections to the notion that plasmodesmata are cytoplasmic canals (Jungers 1930, 1933), and some reservations as to the continuity of the ER through them have been expressed (eg., Porter and Machado 1960; Falk and Sitte 1963). Furthermore, Bisalputra (1966) has stated firmly that the ER does not go through the pits in the walls of some of the algae.

The appearance of the plasmodesmata does depend to some extent upon the fixation procedure employed. In this work, the tissues were fixed in glutaraldehyde/OsO_4 at 0° C, and it seem likely that the "spaces" seen around the central dark core and the "cavity" in the middle of the wall are only apparent spaces, which, *in vivo*, contain material not preserved by these procedures. If so, these connections may not constitute any real "pore" in the wall, for it would be difficult to imagine even a single ribosome passing through the gap. However, these structures may constitute preferential pathways for the transport of molecules from cell to cell or for special types of surface movements because they do represent regions in which the plasmalemma is in contact from one cell to the next.

The nature of the central dark "core" is not clear. It is possible that it is related to a spindle fiber, perhaps "trapped" there during cell-plate formation, or perhaps originating there during the formation of the plate. In favorable views, it does have some substructure in TS (Fig. 12) but the relationship between this element and other microtubules (Ledbetter and Porter 1963; 1964) is a matter for future work.

While there is room for disagreement about the structure of the plasmodesmata, most investigators have commented upon the association between the ER and the plasmodesmata. The type described here has been seen in several other plant systems (e. g., oat leaves, Gunning; *Phleum* roots, Ledbetter; both personal communication; bean leaves, Holowinsky and O'Brien, unpublished). If this type of association should prote to be a general one, it may well correlate with some aspects of tissue polarity.

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Observations on the Fine Structure of the Oat Coleoptile

III. Correlated Light and Electron Microscopy of the Vascular Tissues

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With 40 Figures

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Introduction

Vascular tissues, the xylem and phloem, have fascinated plant anatomists for a long time and by the late 1940's a vast amount of information had been gathered upon the structure, function, comparative anatomy and evolution of these tissues. At about that time, the electron microscope became a serious tool in biological research, and it is scarcely surprising that vascular tissues have been the subject of a considerable number of fine structural investigations. Much of the literature which has accrued from this work is based either upon an examination of walls or wall fragments, or upon material fixed in potassium permanganate or osmium tetroxide (OsO_4). While a certain amount of valuable information has been obtained from these studies, it is self-evident that such specimens show a level of preservation, especially of the cytoplasm, which is markedly inferior to that obtained in tissues prefixed in an aldehyde (Sabatini et al. 1963; see, e.g., Ledbetter and Porter 1963, 1964; Hepler and Newcomb 1964; Gunning 1965 a, 1965 b). If we are to hope to understand the relationship between physiology, biochemistry and fine structure, we cannot afford to base our knowledge of fine structure solely upon results obtained with inferior methods of specimen preparation.

These observations on the vascular system of the coleoptile are presented with this in mind. Arrigoni and Rossi (1962, 1964) have investigated the same tissue in material fixed in permanganate. In addition, Cronshaw and Bouck (1965), Cronshaw (1965) and Pickett-

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Heaps and Northcote (1966) have described some aspects of xylem differentiation in young coleoptiles of oats and wheat, using material fixed in OsO_4 and glutaraldehyde/OsO_4.

In this paper, special attention is given to the structure of the cells as they are found in the mature (25–35 mm) coleoptile. Although the bundles of such coleoptiles contain cells in various stages of differentiation (O'Brien and Thimann 1965), no attempt has been made to study the cytology of differentiation, though a few observations, all on the phloem, are made in passing. It has been possible to confirm many of the observations in the literature and especially those made by Cronshaw and Bouck (1965) and Pickett-Heaps and Northcote (1966). However, many new features have arisen, notably in connection with the structure of the wall of the mature tracheary element, and the fine structure of the parenchyma cells. In addition, a serious attempt is made to relate the results obtained with light microscopy to those obtained in the EM, for it is our belief that when the two methods are used together the results powerfully reinforce one another.

Materials and Methods

The observations which follow are based almost entirely upon coleoptiles of *Avena sativa* L., *Avena fatua* L., *Secale cereale* L., *Zea Mays* L., and *Triticum vulgare* Vill. The fine structural study has concentrated almost entirely on the oats. The coleoptiles were grown to a length of 25–35 mm under the conditions described in detail by O'Brien and Thimann (1965), and the tissues were prepared for electron microscopy by the methods given in detail by O'Brien (1967). In general, the tissues were fixed in glutaraldehyde/OsO_4, dehydrated in an acetone series and embedded in Araldite. In addition, some specimens were fixed in an acrolein/OsO_4 schedule: all subsequent steps were identical. The acrolein/OsO_4 schedule follows:

Tissues were fixed in 10% acrolein in tap water for 12–16 hours (Feder 1960), and washed in 0.025 M. sodium phosphate buffer at pH 6.8 for at least 24 hours, always at 0° C. The washing time was determined in part by the time required to remove the smell of acrolein from the vial. Post-fixation was in 2% OsO_4 in 0.05 M. sodium phosphate buffer at pH 6.8 for 12–15 hours at 0° C. Note: Acrolein supplied by Shell Chemical Co. may usually be used without further purification. However, if it is appreciably turbid or if a 10% solution in tap water has a pH much below 6.5, it should be re-distilled before use. (Caution: Acrolein is very reactive, poisonous, volatile liquid and should be handled with great care, especially during distillation: see Smith 1962, pp. 234—239).

Observations

A. Fresh Tissues and Cleared Whole-mounts

The following summary is based upon sections of fresh coleoptiles, cut by hand and stained with toluidine blue 0 (O'Brien et al. 1964), and upon whole mounts of tissues cleared essentially by the method of Simpson (1930). A more detailed treatment has been published previously (O'Brien and Thimann 1965; Thimann and O'Brien 1965).

(i) The bundles of the coleoptile in the mature embryo are entirely provascular, but differentiation of the xylem is extremely rapid after germination. At first, tracheary elements differentiate acropetally and mature tracheary elements are present in the tip of a coleoptile which is only 1.5 mm long. Thereafter, differentiation is basipetal and apparently continues throughout the life of the organ, but there are always more files of tracheary elements in the uppermost reaches of the bundle than there are at the base.

(ii) The extreme apex of the organ (the uppermost 0.25 mm) is free of vascular tissue: the bundles (usually 2) reach just to the level of the top of the air space. They terminate in a cap of tracheary elements. The arrangement of the apical ends of the bundles varies considerably with the genus.

(iii) Sieve elements are not present in the extreme apex, but do occur about 0.25 mm from the tip of the bundle. The time-course of their differentiation is not known.

(iv) In maize, oats and wheat, the uppermost reaches of the bundles are amphivasal, the xylem surrounding the phloem in a ring. This condition persists throughout the length of the bundle in maize but only for the most apical millimeter or so in wheat and oats.

(v) A lacuna, formed by the destructive stretching of the first-formed tracheary elements, occurs at all levels below the first 3–5 mm. This is mirrored on the phloem side by a metachromatic mass of material which seems to have been formed by stretching and crushing of the early phloem cells.

(vi) The coleoptile bundles are not delimited by a classical bundle sheath, although some of the xylem and phloem parenchyma of the basal part of the oat and wheat coleoptiles may lignify and form a remnant of such a sheath. See also Avery 1930.

B. Light Microscopy of Thin Sections

Figs. 1 and 2 are light micrographs of the upper part of vascular bundles in the oat coleoptile: the tissue was fixed in acrolein, embedded in glycol methacrylate, and sectioned at 1.5–4 μ (for methods, and a general discussion of the histology of this region see O'Brien and Thimann 1965; Thimann and O'Brien 1965). Fig. 1 is a montage of the upper part of the bundle in LS and Fig. 2 shows the appearance of the bundle in TS at a level about 1.5 mm from the coleoptile apex. Figs. 3 and 8–11 illustrate the cytology of the tissue in greater detail. Five kinds of cell are present, belonging to three cell-types; tracheary elements (some are still in the course of differentiation), sieve tubes, and three kinds of parenchyma cell.

(i) The tracheary elements. These are usually vessels, for the cross walls between contiguous elements are at least partially perforated (Fig. 11). The pattern of the lignified thickenings of the wall reflects the conditions of growth of the elements at the time the wall was deposited. Thus, in the apex, where growth is minimal, the cells are reticulately-thickened, while in the

growing regions annular or branched thickenings are the rule. These patterns are modified, of course, by passive stretching (see also Goodwin 1942) and tyloses from neighboring parenchyma cells may invade the lacuna created by the destructive stretching of those cells which differentiated first (Fig. 10).

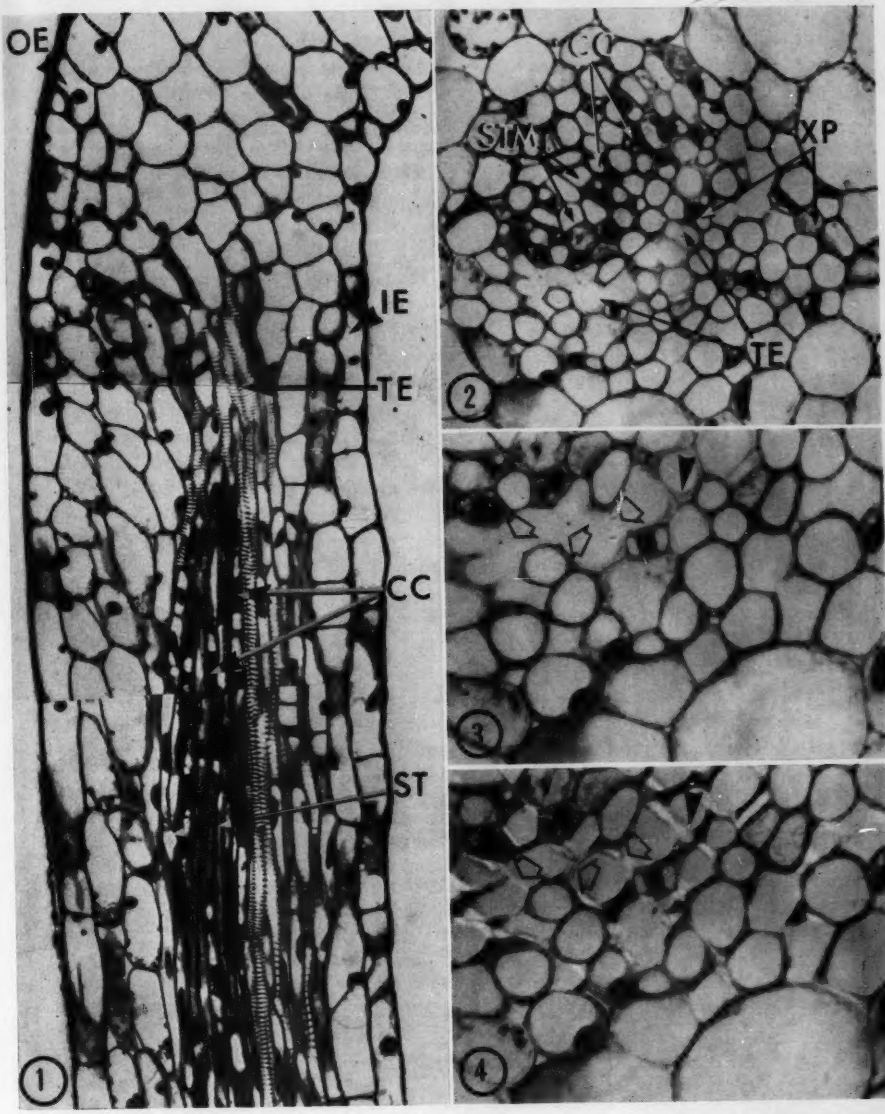
In thin sections, the walls of the tracheary elements, especially in the upper part of the bundle, display a rather unusual image. In many instances, one cannot see any trace of stain in the region which corresponds to the position of the primary wall (Fig. 3, open arrows). In such cases, the arcs of lignified wall appear to be unsupported, and the picture is made even more difficult to understand in those instances where the intercellular material (which takes the place of an air-space where three xylem cells abut one another) is retained (see also Sorokin 1958). In section, this intercellular material is roughly triangular in outline and stains strongly and metachromatically with toluidine blue 0; the color contrast with the green-stained layers of secondary wall (lignin stains green with toluidine blue 0) is most striking in the microscope. Precisely the same phenomena may be seen in sections stained by the PAS-procedure (see Jensen 1962). The intercellular material and the lignified secondary wall are both strongly PAS-positive (this PAS-positivity of the lignified wall is in itself of some interest and is treated in the discussion), but no trace of PAS stain may be detected in those parts of the primary wall and middle lamella region which fail to stain with toluidine blue 0 (see Figs. 16 and 18, insets).

While it was not surprising that the perforation plate of vessel elements failed to stain, it did not seem reasonable to believe that islands of thickening and intercellular material could persist in a vessel element, free of any apparent connection with one another or with the walls nearby. In the EM such walls were found to contain a mat of fine fibrils which were connected to the primary wall beneath the bands of thickening (see below), and when such "hydrolyzed walls" were examined in polarized light, some of the areas which failed to stain were weakly, but definitely, birefringent (Fig. 4, open arrows). This suggested that the primary wall of these cells consists of a residue of cellulose fibrils, free of PAS-positive polysaccharides and free of polyuronides with carboxyl groups available.

Fig. 1. Montage of a longitudinal section (*LS*) of the upper part of a vascular bundle in *Avena sativa*. ×200.

Fig. 2. Transverse section of a vascular bundle in *Avena sativa*, taken about 1 mm from the apex of the coleoptile. ×325.

Figs. 3 and 4. Part of the same vascular bundle as shown in Fig. 2. The open arrows point to areas of "hydrolyzed wall" (see text). Both sections were stained with toluidine blue 0, but Fig. 4 has been photographed under crossed polarizers. Note the weak, but definite, birefringence, at the positions marked by the open arrows, and contrast this with the unstained appearance of the corresponding positions in Fig. 3. The solid arrows show the appearance of the thickened walls under the same conditions. ×1,050.



Figs. 1–4.

This suggestion raised a problem, for it is commonly assumed that cellulose is PAS-positive (Jensen 1962). This point was tested by an examination of the fibres which lie above the vascular bundles in the leaves of the screw-pine, *Pandanus utilis.* These fibers have very thick secondary walls, which, in the early stages of differentiation, are completely unlignified and brilliantly birefringent (Figs. 5 and 6). They give only a faint stain with toluidine blue 0, which suggests that they are low in polyuronides with free carboxyl groups. The compound middle lamella of the fibers and the adjacent primary walls of the parenchyma cells do stain intensely with toluidine blue 0 (Fig. 5). These walls are ideal for testing the PAS-positivity of a wall which is essentially cellulose, and is free of lignin. The wall was found to be PAS-negative (Fig. 7) when the hydrolysis time in 1 % periodic acid was 15 mins (section 2 μ thick). This time is adequate to stain the compound middle lamella of the fibers and the primary walls and starch grains of the adjacent parenchyma cells (Fig. 7, large arrows) and is also adequate to stain the primary walls of roots (Jensen 1962). It is concluded that pure cellulose is PAS-negative, or at best weakly PAS-positive. Thus, the absence of PAS-positivity in the residual material of the hydrolyzed wall in the coleoptile vessel, and its birefringence, are both consistent with the suggestion that this material consists largely of cellulose. An attempt was made to stain this material with the chlor-zinc iodide reagent (Jensen 1962) but the intensity of the stain was too weak to make a definite decision. Curiously, the lignified wall of the element did stain: this is contrary to the general belief that lignified walls do not stain with this reagent, and this point is also taken up in the discussion, along with the PAS-positivity of the secondary wall.

(ii) Sieve tubes. The sieve tube members have sieve plates on their end walls but the orientation of these plates may vary from transverse to almost vertical, even in closely adjacent sieve tubes (Fig. 8, open arrow). In addition, the lateral walls have numerous sieve fields (Fig. 8, large black arrows), which may lie between two sieve-tube members, or between a

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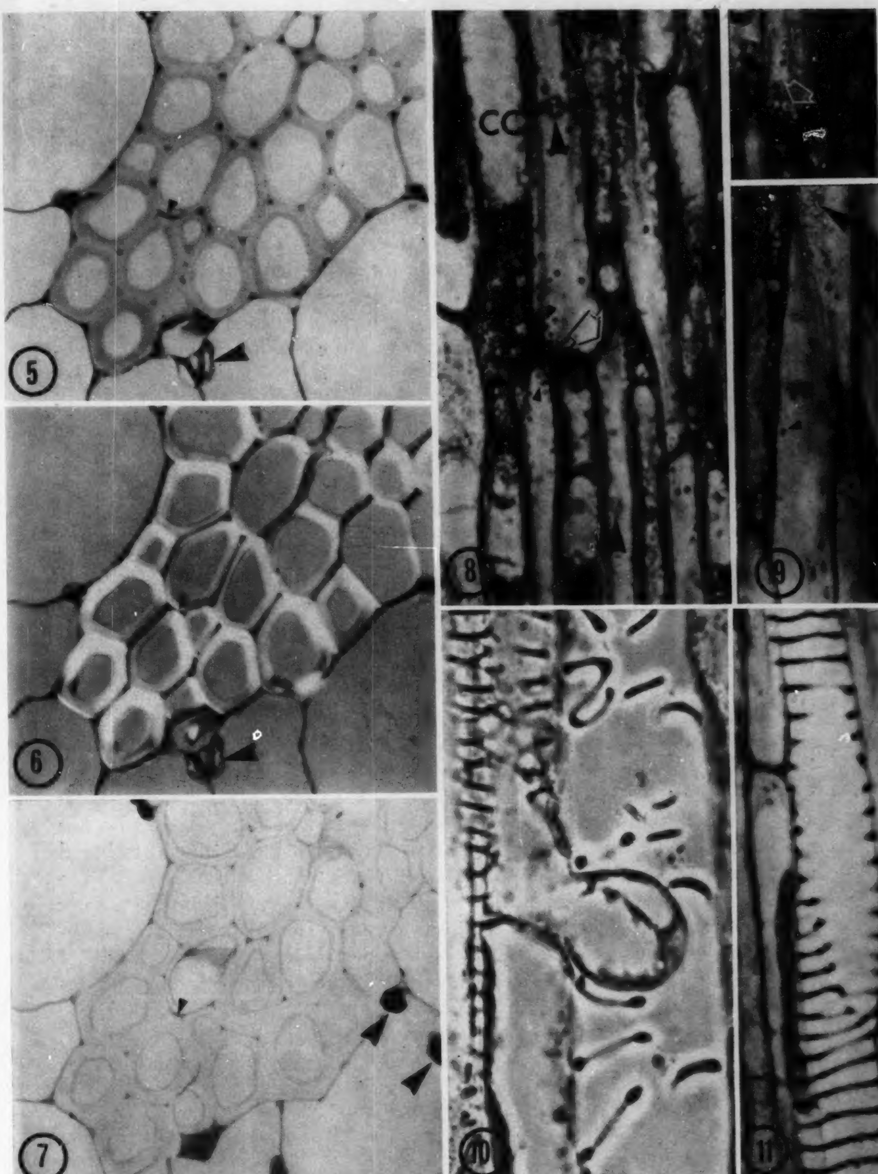
Figs. 5–7. A demonstration of the PAS-negative behavior of cellulose in fibers of *Pandanus utilis,* the screw-pine. Fig. 5 is stained with toluidine blue 0, Fig. 6 is the same section under polarized light, and Fig. 7 is a closely adjacent section stained by the PAS-procedure (1% periodic acid for 15 minutes). The large arrows point to starch grains (which are essentially unstained by toluidine blue 0, but stain very intensely with PAS), and the small arrows point to the same region in the middle lamella of the wall. It is quite clear that the secondary wall is almost unstained by toluidine blue 0 (Fig. 5), strongly birefringent (Fig. 6), and PAS-negative (Fig. 7). ×1,050.

Figs. 8 and 9. *LS* through the phloem, stained with toluidine blue and acid fuchsin. The open arrows point to the sieve plates (Figs. 8 and 9, inset), the large black arrows to sieve fields on the lateral walls of the sieve tube members. The small black arrows mark the characteristic sieve-tube granules. All ×1,050.

Figs. 10 and 11. *LS* through the xylem. Fig. 10 shows the stretched protoxylem elements and a tylosis from a neighboring parenchyma cell. Fig. 11 shows the characteristic vessel perforation-plate in LS in these elements. Both ×1,050.



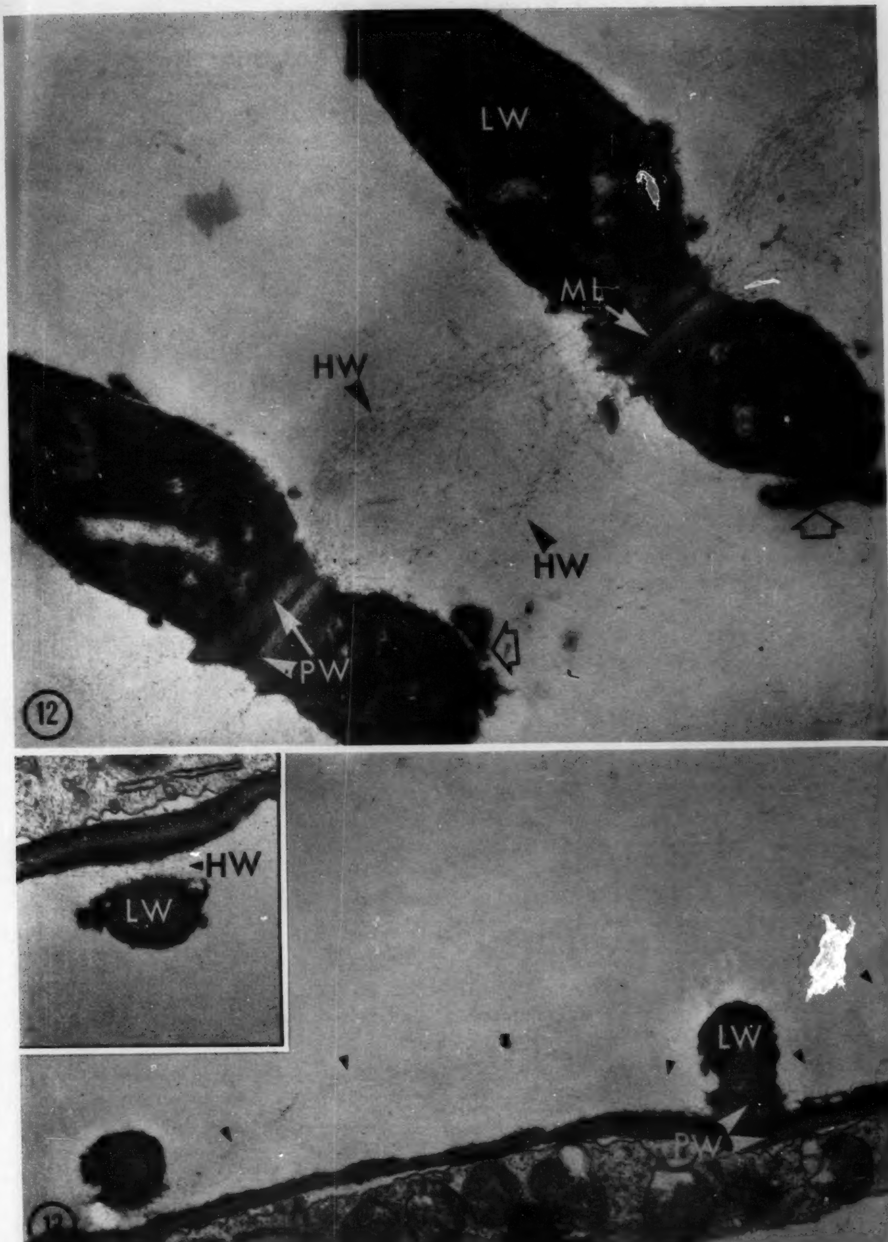
Figs. 5–11.

sieve-tube member and a parenchyma cell. The primary wall region of the sieve tubes stains intensely metachromatic with toluidine blue 0 and since the "pores" of the sieve plates and sieve fields fail to stain with this dye, the "sieve-like" nature of these structures is most obvious, especially in surface view (Fig. 9, and inset). The inner layers of the sieve-tube wall stain weakly both with toluidine blue 0 and PAS, but they give a strong reaction with chlor-zinc iodide and are strongly birefringent; it may be deduced that they are richer in cellulose than the outer layers of the wall.

The "cytoplasm" of the sieve tubes stains poorly, except in the region of the sieve plates which show an aggregation of amorphous material of high acidophilia. In addition, the tubes are rich in acidophilic granules the majority of which appear to consist of an aggregate of 3–6 particles, while the remainder consist of units of these aggregates (Figs. 8–9, small arrows). These granules are very evident in phase contrast, even in unstained material, and may be detected in differentiating sieve elements before the nucleus and cytoplasm complete their differentiation (Fig. 26). The granules (which are derived from the plastids: see below) often collect in the mass of acidophilic material which occurs at the sieve plate. In fresh material, structures which resemble these granules, as well as material which is closely applied to the sieve plates and the sieve fields, stain intensely with sudan black B. This lipophilic material is not retained through the acrolein/methyl cellosolve/glycol methacrylate procedure used here, and its significance cannot be assessed at this point.

(iii) The parenchyma cells. Interpretation. These fall into three categories: (a) basophilic cells, (b) cells which stain strongly with both acidic and basic dyes, and (c) cells which stain weakly with either dye. In the earlier study (O'Brien and Thimann 1965) is was pointed out that it was difficult to classify these cells. This difficulty arises in part because the earlier and definitive work on the differentiation of monocotyledonous bundles (e.g., Esau 1943) used material fixed and stained by procedures which produce an image quite different to that seen here, making direct comparison difficult. Secondly, the ontogeny of the coleoptile bundle has not been worked out in detail and thus one cannot make diagnoses based upon the spatial relationships of the cell-types. This is particularly true in the upper part of the bundles, which tend to be amphivasal; one may find tracheary elements which abut sieve tubes. However, electron microscopy and further light microscopy have shown that, contrary to the earlier suggestion (O'Brien and Thimann 1965), the cells which are both in-

Figs. 12 and 13. *LS* of the mature vessel elements. Fig. 12 shows the lateral wall between two adjacent elements. The bands of thickening (*LW*) are attached at their bases to a clear-cut primary wall. Between the bands pass files of fibrils, the remains of the hydrolyzed wall (*HW*). In Fig. 13, the vessel element has been stretched (note the distance between the rings) and the hydrolyzed wall is scarcely visible. Its position has been marked by the small arrows. The inset on Fig. 13 shows that the hydrolysis may sometimes include the primary wall beneath the thickening. The open arrows in Fig. 12 indicate cytoplasmic remains. Fig. 12: ×28,400. Fig. 13: ×17,200. Inset: ×21,300.



Figs. 12 and 13.

tensely acidophilic and basophilic are *not* differentiating sieve elements. The interpretation of the vascular parenchyma is as follows.

The basophilic cells are most often xylem parenchyma: this is most clear where the cells lie between the tracheary elements and the wider cells of the extrafascicular parenchyma. However, basophilic cells also occur near sieve tubes, and in some cases it has been possible to show that such cells are contained within a vertical file of cells which contains a differentiating sieve element (Fig. 26). It has also been possible to show that those differentiating sieve elements which can be identified positively by the presence of the refractile granules are not particularly acidophilic (see also Fig. 28).

The cells which stain intensely with acidic and basic dyes are best interpreted as companion cells (Fig. 31).

Weakly-staining cells occur at the margins of the bundle, especially on the side near the outer epidermis. These cells develop a thicker wall and in the lower reaches of the bundle may become lignified.

Cytology. The basophilic xylem parenchyma cells afford one a rare opportunity to resolve the endoplasmic reticulum readily and consistently in a stained section (Fig. 32, inset). In thick sections (4 μ) one may focus up and down through the skeins of cisternae which are most obvious in the aggregates of cytoplasm near the cross walls in these cells. The nuclei are elongated and moderately chromatic, and there is an abundance of mitochondria, which stain strongly with acid fuchsin and may be readily resolved in phase contrast (Fig. 32, inset).

The companion cells of these bundles have the most highly-stained cytoplasm of any mature cell-type in the coleoptile. The nuclei are elongated, highly chromatic, and contain a prominent nucleolus. The parietal cytoplasm contains an abundance of bodies about 1 μ in diameter, which fail to stain (Fig. 31). These bodies are in fact mitochondria (see below), but they have lost their characteristic acidophilia. This point will be discussed further after the fine structure has been presented.

The weakly-stained cells show no special features of cytological interest beyond those which are common to parenchyma cells in an apparently low state of metabolic activity.

C. Fine Structure

(i) The mature trachery elements. Fig. 12 shows part of the wall between two vessel elements, seen in LS. The bands of lignified secondary wall are cut in TS and reveal a certain amount of pattern in their substructure. The electron-dense material is very finely textured and it is presumably either lignin or the associated hemicelluloses which have "stained." The bands of secondary wall are joined at their bases by two strips of primary wall, between which may be seen the slightly darker region which cor-

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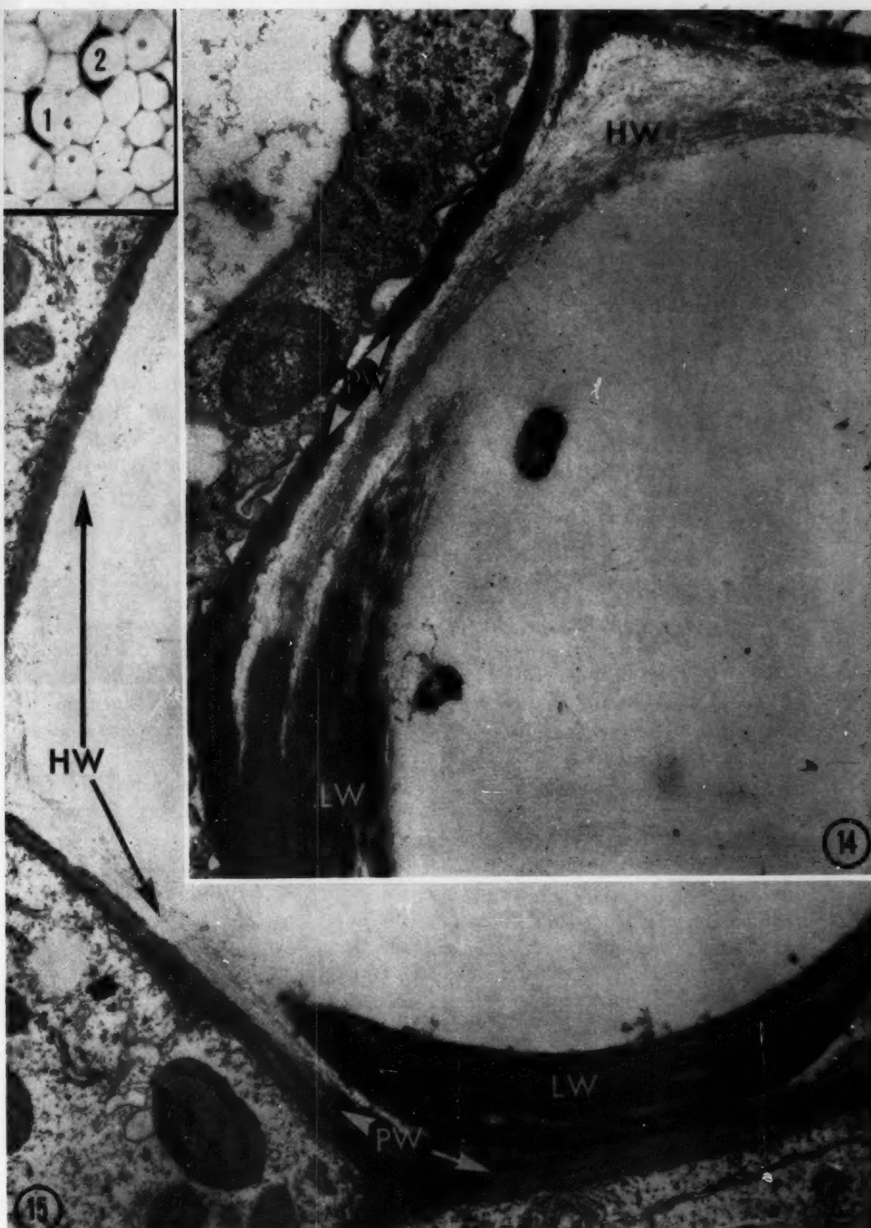
Figs. 14 and 15. Vessel elements in TS. The inset to Fig. 15 is a light micrograph of the adjacent section to Figs. 14 and 15, stained with the PAS procedure. Cell 2 is in Fig. 14, cell 1 in Fig. 15. The nature of the hydrolyzed primary wall is evident. Fig. 14: $\times$26,000. Inset: $\times$1,050. Fig. 15: $\times$15,600.



Figs. 14 and 15.

responds to the middle lamella. Remnants of cytoplasm may also be detected on the bands (Fig. 12, open arrows). At first sight, these bands of secondary wall seem to be "free in space" but on closer examination one may detect files of finely fibrillar material which pass from one remnant of primary wall to the next. These bands of fibrils seem to be continuous with the primary wall and hereafter, these fibrillar remains will be called a "hydrolyzed wall" (HW in Fig. 12). In some cases (Fig. 13 and inset), the primary wall is hydrolyzed beneath the thickened rings on both sides of the cell, and the ring thus appears to be free. It may be significant that such free rings are usually tilted out of the plane of the other nearby rings which are still attached by a non-hydrolyzed wall.

The phenomenon is equally striking in TS (Figs. 14–18). Again, the primary wall beneath the rings (which may appear as arcs in slightly oblique TS) is replaced by a fibrillar mat in the region away from the rings, and occasionally even the zone beneath the ring has been hydrolyzed (Fig. 16). Figs. 17 and 18 illustrate two other cases in which both primary walls of adjacent vessel elements have been hydrolyzed. In a stained section of such a wall-junction in the light microscope (Figs. 17 and 18, insets), no material can be detected but, as was pointed out above (Fig. 4), the fibrillar component of such a wall is birefringent, and probably contains cellulose.

Fig. 18 shows a vessel element in what appears to be an advanced stage of cytoplast and wall disintegration; only a few remnants of membrane and a few ribosomes are left. We have already suggested that the crystal-containing bodies may be lysozome-like entities in the parenchyma cells (see O'Brien and Thimann 1967; and Cronshaw 1964) and it was no surprise to find such a structure, free of any detectable enclosing membrane, in an element in an advanced stage of degeneration. It seems possible that these organelles contain the enzymes responsible for the destruction of the cytoplasm and perhaps they also contain the enzymes which must be responsible for the hydrolysis of the non-cellulosic polysaccharides of the primary wall.

(ii) The mature sieve-tube member. The structure of the mature sieve-tube member (STM) differs in several respects from any other cell in the bundle. Engleman (1965) coined the term "mictoplasm" to describe the mixture of cytoplasm and vacuole which is generally believed to make up the contents of functioning sieve tubes, and this term will be used here. In these cells, the mictoplasm consists of an extremely thin parietal layer, bounded by a definite plasma membrane which stains darker on the side facing the wall (Figs. 20, 21, 23, and 39). Mitochondria are moderately

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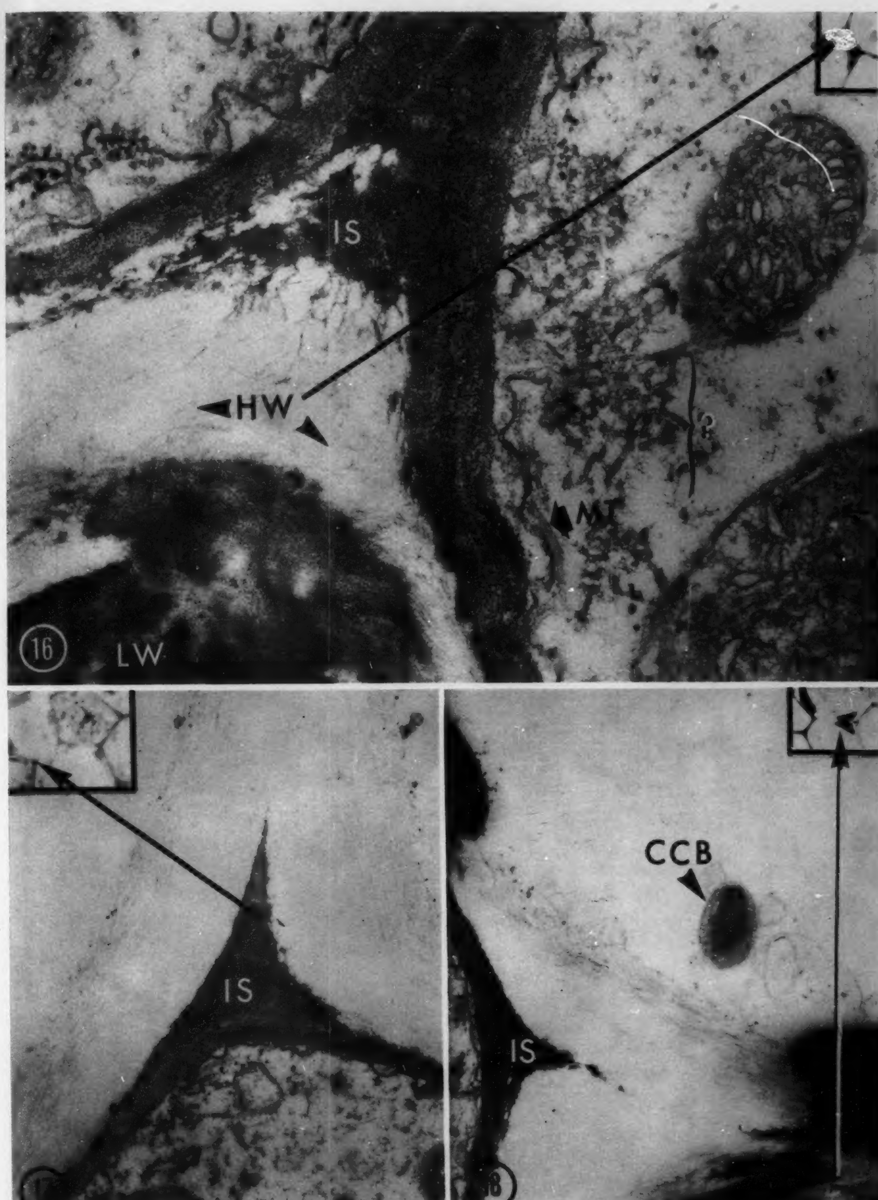
Figs. 16–18. Various appearances shown by the hydrolyzed walls. Note that the intercellular material may be hydrolyzed (Fig. 16), or remain essentially intact (Fig. 17). In each case, the inset is the adjacent section, photographed in the light microscope. The nature of the mass of tubules at ? in Fig. 16 is obscure. Fig. 16: ×32,000; inset: ×1,050 (PAS-stain). Fig. 17: ×26,000, inset: ×1,050 (Methylene blue/Azure II stain). Fig. 18: ×21,400; inset: ×1,050 (PAS-stain).



Figs. 16–18.

abundant in this parietal layer and, contrary to the findings of some authors in other tissues, appear to be reasonably normal (Figs. 19 and 20). The acidophilic granules, diagnostic for the sieve tubes in the light microscope, are seen to consist of aggregates of crystalloids, and the aggregates are usually surrounded by a double membrane (Figs. 20 and 21). Individual crystalloids do occur free in the mictoplasm (Figs. 19 and 21). Among the most interesting components of the mictoplasm are the bundles of filaments which often occur near the sieve plates or sieve fields. The general structure of these aggreates is remarkably similar to those described in the margins of the vacuole in parenchyma cells (see O'Brien and Thimann 1967). Usually, long filaments, about 220–240 Å wide, which bear a superficial resemblance to microtubules (Ledbetter and Porter 1963) appear to radiate from a tangle of finely fibrillar material which, in sieve fields, may occlude the "pores" on the STM-side (Figs. 20, 23 and 25).

Stacks of a cisternal element which has been termed the sieve tube reticulum (Bouck and Cronshaw 1965) have not been seen in this material, but the parietal mictoplasm does contain a single cisternal element which may be the form of this system in this tissue (Fig. 21).

The cell wall offers several features of interest. It is considerably thicker than the wall of adjacent parenchyma cells and in addition to the outer layers (which are similar in structure to the wall of the parenchyma cells) there is an inner layer of irregular thickness which has a loose fibrillar texture (Figs. 19–21, 23 and 25); this material is also deposited in the region of the sieve plate (though not at the "pores") and its fibrillar component has a considerable affinity for the electron stains. It seems likely that this material is the "nacré" which is characteristic of the sieve-cell wall in many other species (see Esau and Cheadle 1958).

The sieve plates and sieve fields deserve special comment since the wall is distinctly modified in these regions. In TS, the sieve plate is seen to consist of ribs of material which are indistinguishable in fine structure from the wall in other parts of the cell. Presumably, it is this component of the sieve plate which stains so readily with toluidine blue 0, as does the outer layer of the wall. These ribs of apparently unaltered wall intercalate with regions of extremely low electron-contrast. On the mictoplasm side, such areas of low contrast appear to be limited by a membrane, which is not demonstrably different from the plasmalemma in other parts of the cell (Fig. 21, small arrows; Fig. 23). However, it has *not* been possible to prove rigorously that the plasmalemma is continuous through the plate. However, the areas which are surrounded by this material of low electron contrast do appear to constitute a membrane-lined "canal", within which occurs material whose texture closely resembles that of the nearby crystalloids (Fig. 21).

The sieve fields between a STM and a parenchyma cell have a somewhat different structure. On the STM-side, the structure resembles that of a small sieve plate, except that the "pore" in the wall seems to be occluded by tangled skeins of material which are associated with the filamentous structures (Figs. 23 and 25). The structure of the wall has again been modi-

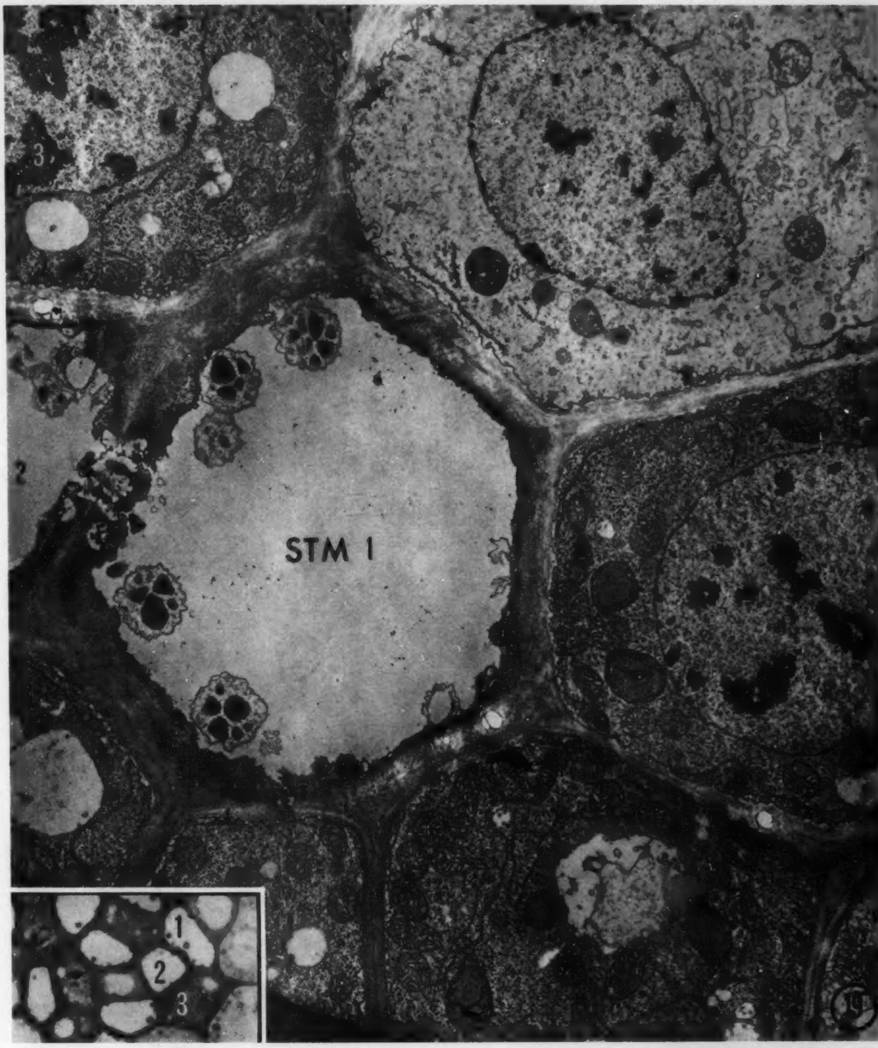


Fig. 19. Low magnification picture of the phloem. The inset shows a closely adjacent area, stained for light microscope by the methylene blue/Azure II procedure. The cells numbered in the inset are also numbered in the micrograph. ×9,100, inset: ×1,050.

fied and material of low electron-contrast surrounds the "pores." On the parenchyma cell side, the structure closely resembles that seen in plasmodesmata in similar parenchyma cells (cf. Figs. 23 and 25 with Fig. 24), and they display a similar relationship to the ER.

(iii) Differentiating sieve elements. The sequence of differentiation has not been studied in any systematic way in the coleoptile, but a few observations are noteworthy. In the early stages, there is no way to distinguish a differentiating sieve element from any other basophilic parenchyma cell (Figs. 26 and 27). This continues to be the case for cytoplasmic characteristics even when it is clear from the structure of the wall that the cell is to become a sieve element (Fig. 27). As was found to be the case in pine (Srivastava and O'Brien 1966) the first definite change in the cytoplasm seems to take place in the plastids and from that time, the cell apparently moves repidly towards the mature state. Fig. 28 shows two elements, the upper of which has an essentially mature mictoplasm, while the lower one still has a clearly defined tonoplast but the plastids have developed the crystalloids which are characteristic of the mature cell. The differentiating cell shown in Fig. 27 is probably at a stage just a little earlier than shown in the light micrograph of Fig. 26, in which one can certainly detect the crystalloids of the plastids by phase contrast. It is also important to note that the cytoplasm is not acidophilic (Fig. 26) and its fine structure is quite different from that of the acidophilic cells (an acidophilic companion cell, CC, abuts the differentiating sieve element in Fig. 28). The cross wall between the elements in Fig. 28 is interesting, for it is clear that the region around the plasmodesmata has undergone an alteration, and consists of the material of low electron contrast which is present at maturity. It certainly appears that the formation of the plate involves an alteration of the wall content and structure in the region of the plasmodesmata (see also Bouck and Cronshaw 1965; Wark and Chambers 1965; Esau 1965 a; 1965 b; and the illustrations provided by Newcomb in the paper of Albersheim 1965).

A late stage in the development of the nacré wall is shown in Fig. 29. The cytoplasm is very rich in polyribosomes at this stage and the asymmetric staining of the plasmalemma is particularly evident.

Figs. 20 and 21. A TS of a STM, showing the structure of the sieve plate and the contents of the cell. The inset of fig. 20 (stained with methylene blue/Azure II procedure) shows a closely adjacent section and establishes the nature of the sieve tube granules. These modified plastids sometimes rupture and release free crystalloids (*CR*) into the mictoplasm. Note the aggregations of filamentous material (?) in the mictoplasm. Material whose electron contrast resembles that of the crystalloids appears to plug the pores between the adjacent STM. The plasmalemma is clearly evident (arrows, Fig. 21) and the altered nature of the wall around the pores is most striking (*). Fig. 20: ×18,900, inset: ×1,050. Fig. 21: ×3,600.

Fig. 22. Part of a nuclear remnant in the mictoplasm of a STM in the coleoptile of rye. The remnant seems to consist chiefly of a dense aggregate of chromatin. ×28,000.

Figs. 20–22.

All of the major features of the structure of the mature STM seen in oat coleoptiles could also be seen in the STM of rye coleoptiles; in addition, on one fortunate occasion, a section passed through the remains of a nucleus, clearly in an advanced stage of dissolution (Fig. 22). It appears that pieces of the densely-packed chromatin may resist dissolution in the mictoplasm and it is possible that it is this material which is responsible in part for the reports of "extruded nucleoli" in mature STM (Esau 1965 a).

(iv) Xylem parenchyma. In the light microscope, these cells are rich in ER and mitochondria and this may be seen in greater detail at the EM level in Figs. 30, and 32–34. In addition, these cells show several cytological features of interest. First, a good deal of the chromatin is associated with the nuclear envelope, whose outer layer is commonly invested with ribosomes (Fig. 32). In both of these respects, these cells differ from what has been observed in the extrafasicular parenchyma and epidermal cells (see O'Brien 1967; O'Brien and Thimann 1967) and it seems possible that the two phenomena are correlated. Aggregates of rough ER, such as those seen in Fig. 34, clearly explain how one may see skeins of stained material in the light microscope (Fig. 32, inset), for the surface of these membranes is crowded with ribosomes, many of which are arranged in the polyribosomal form (see also Bonnett and Newcomb 1966). The mitochondria have a very dense stroma (especially after acrolein fixation, Fig. 34), numerous dense granules and well-developed cristae. The ground substance of the cell is rich in free ribosomes (again chiefly in aggregates) and also contains droplets of material which are presumed to be lipid.

The plasmodesmata show the same basic structure and relationship to the ER as has been described in detail for the extrafascicular parenchyma cells (see O'Brien and Thimann 1967) is clear that the secondary wall of tracheary elements may sometimes be deposited on top of what was a plasmodesmatal connection between the elements (Fig. 32; cf. Cronshaw and Bouck 1965).

Microtubules are abundant in the cortices of these cells but are often difficult to resolve because of the electron density of the ground substance. More important, the cell cortex seems to be drastically modified in many instances in the regions which underly a hydrolyzed wall. Any regular

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Fig. 23. A sieve field between a parenchyma cell and an STM. Note the altered wall material (*) on the STM side, and the resemblance to plasmodesmata on the parenchyma cell side. The unit membrane which lines the pores of these plasmodesmata is clearly demonstrated (arrow). The filamentous material (?) which occurs in the mictoplasm seems often partly to occlude the pores of these lateral sieve fields. ×83,300.

Fig. 24. A plasmodesmatal connection between two parenchyma cells in the phloem. The structure is identical to that seen in extrafascicular parenchyma. Again, there is the same close association with the ER, which has a particularly dense content. ×64,000.

Fig. 25. Another sieve field in which the contrast between the plasmodesmatal nature of the pit on the parenchyma cell side and the sieve like structure of the STM side is most marked. The asterisk* again shows the modified wall material. ×40,000.

Figs. 23–25.

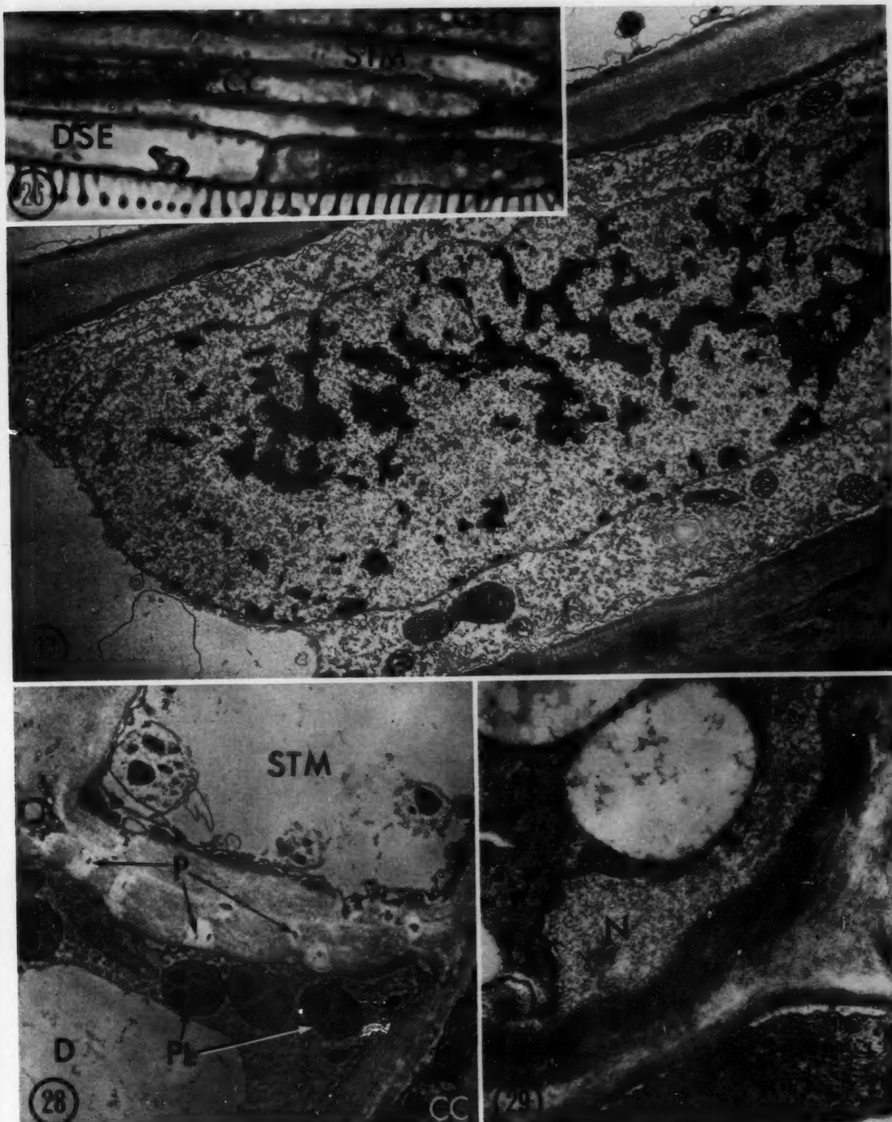
orientation of the microtubules (which may have existed when the cells were younger; Cronshaw and Bouck 1965) seems to have been profoundly disturbed. In addition, aggregates of filamentous structures, again reminiscent of the material in the mictoplasm of the sieve tubes, or of the vacuole and pit fields of the parenchyma cells are commonly present (Figs. 16–17). The presence of such structures at the margins of these parenchymal cells is of some interest because it is these cells which can invade the lumen of the stretched protoxylem and form tyloses (Fig. 10).

The other type of modification of the margin of the parenchyma cells is shown in Figs. 33 and 35. This activity is again always confined to parenchyma cells adjacent to a tracheary element but it has also been observed in companion cells (Fig. 35) when they abut such an element. It does not occur in all of the parenchyma cells, but is does occur in other genera (Fig. 40). It is tempting to suggest that this appearance of the wall/cytoplasm interface may reflect some type of pinocytotic activity, perhaps connected with the removal of the degraded cell contents from the tracheary element.

(v) The companion cells. In many ways these cells are the most remarkable of any within the bundle, for the density of the ground substance attains a level never recorded in a plant cell. The ground substance contains an almost close-packed array of ribosomes which are embedded in a dense felt of fibrillar material (Figs. 35 and 36). In addition, long cisternal elements of rough ER course through the cells, but the cisternae appear to be slightly swollen, and contain some electron-dense material. At maturity, the mitochondria appear to lose their stroma, and it is quite clear that it is these degenerated mitochondria which correspond to the numerous "clear" bodies of the cytoplasm of these cells when seen in the light microscope (Fig. 31). Whether the dramatic enrichment of the ground substance with acidophilic material is in fact due in part to the massive release of the mitochondrial stroma material is not known, but it is certainly suggestive (cf. Figs. 35 and 36).

The nucleus of these cells contains extremely dense chromatin. Unfortunately, a full discussion of the fine structure of the nucleus is beyond the scope of the present investigations (see Moses 1964 for a critical discussion), but it can be reaffirmed that the fine structure of the nucleus and the state of differentiation of the cytoplasm are inextricably linked. Clearly, these companion cells are no exception.

Figs. 26–29. Various stages in the differentiation of sieve tubes. Fig. 26 is a light micrograph showing the crumpled nucleus and characteristic granules of a stage just a little later than that seen in Fig. 28. Fig. 27 is an EM of a very early stage in which the only obvious sign of differentiation is the thickened wall. Fig. 28 shows adjacent elements, one of which appears to be mature, while the lower cell still retains its tonoplast. However, the differentiation of the plastids is evident. Note the alteration in the wall in the region of the plasmodesmata. Fig. 29 is a late stage in differentiation and shows the characteristic nacré wall (*N*). The tonoplast is still intact. Fig. 26: ×1,050. Fig. 27: ×8,400. Fig. 28: ×21,000. Fig. 29: ×26,000.



Figs. 26–29.

The marked acidophilia of the cytoplasm of these cells is obviously correlated with the electron density of the ground substance and it is impossible to decide whether these cells have microtubules or not.

(vi) Weakly basophilic parenchyma cells. In the light microscope, these cells, which are most common in the phloem on the side of the bundle near the outer epidermis, are similar in their staining reactions to the extrafascicular parenchyma. This is borne out in the electron microscope, which shows that their fine structure is indeed similar to that of the parenchyma cells, except for the poorly developed plastids, a phenomenon which they share in common with the other vascular parenchyma. The cells shown in Fig. 37 lie 16 mm from the apex of a 35 mm coleoptile and it is possible that the electron density of the inner layers of the wall is due to the development of lignin.

(vii) The protophloem. Close to the weakly-basophilic parenchyma cells lies the region which, below the uppermost 3–5 mm of the coleoptile, is occupied by a mass of metochromatic material (Fig. 38). This substance is usually interpreted as the crushed remains of the protophloem (Esau 1965 a). It was in this region that the cells shown in Fig. 39 were encountered, again 16 mm from the apex of a 35 mm coleoptile. It is difficult to be certain about the nature of the upper cell, but since it has ribosomes in its cytoplasm, it may be a companion cell. There can be no doubt that the lower cell is a STM, because of the characteristic granules in the plastids. In both cells, the plasmalemma has begun to separate from the wall, which seems to have swollen and has encroached upon the lumen of the cells. The mictoplasm of the STM is full of similar fibrillar material but it has a somewhat different electron density. The mitochondria of both cells seem to be badly disorganized.

It seems likely that the metachromatic mass of material which comes to occupy the position of the protophloem in primary bundles is derived from this wall material and it would appear that both companion cells and STM contribute to it. It would be most interesting to known whether this material is synthesized at this time or whether it develops from existing wall material. Is this the last synthetic act of the STM companion-cell association?

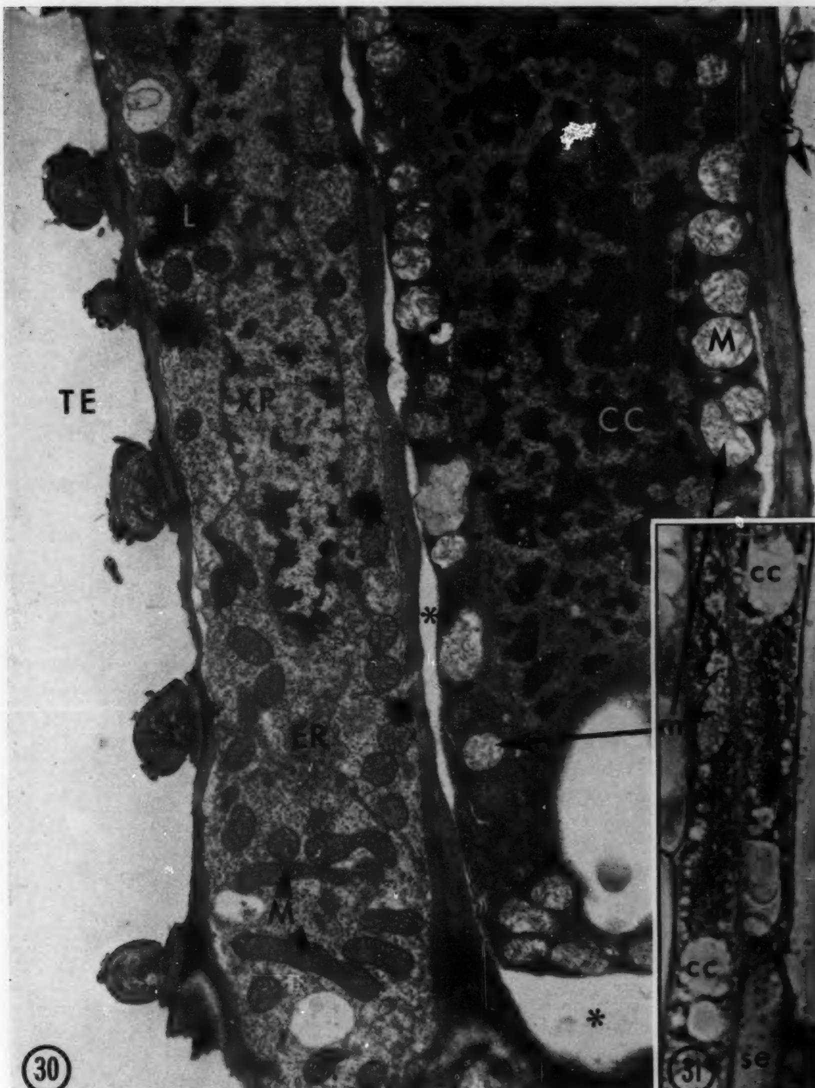
Discussion

A. The Tracheary Elements

The hydrolyzed walls of the perforation plates and the lateral walls of these vessel elements raise several points. First, it is clear that the dissolution of the non-cellulosic polysaccharides of these primary walls must be enzymatic, and although it may occur in a region in which the cells are growing, it may also occur in the extreme apex of the bundle where elongation does not take place. Roelofsen (1965), in an excellent review, has

Fig. 30. LS of the parenchyma cells of the vascular system. ×10,900.

Fig. 31. Light micrograph of the companion cells, showing the highly chromatic nucleus, and the unstained mitochondria. ×1,050.



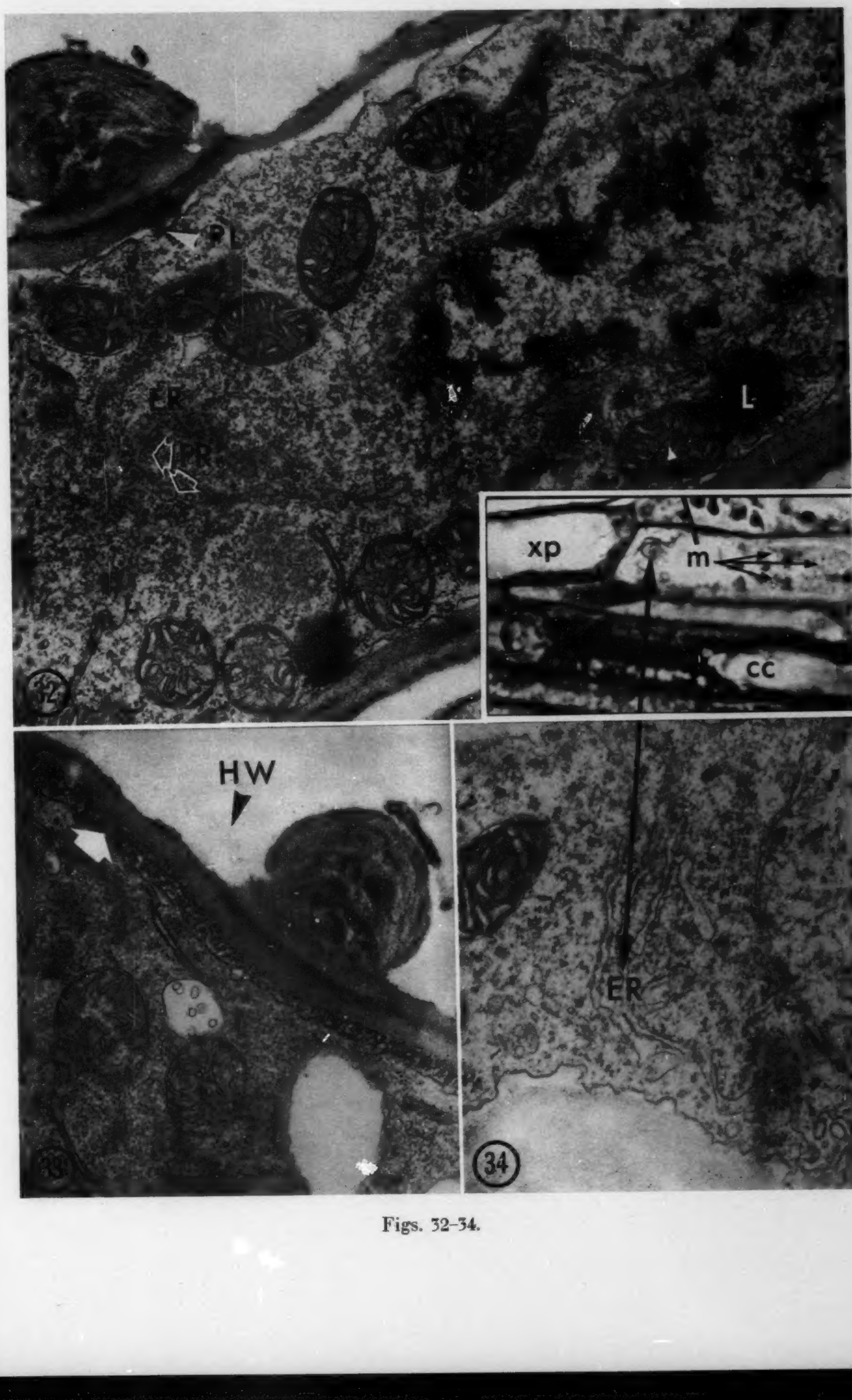
Figs. 30 and 31.

recently stated that "breakdown of cell wall substance in primary walls, going beyond the plasticization of the amorphous interfibrillar substances, has, in our view, not been proven to exist." The observations reported here go far towards providing such a demonstration. They also suggest that it is the differentiating cells themselves which are the loci of hydrolase production. It is of course not excluded that the surrounding cells could supply the enzymes to the differentiating vessel elements without digesting their own wall (e.g., by releasing them at plasmodesmata, or by releasing them as zymogens which might be activated in the differentiating cell). However, since the differentiating cells do contain the crystal-containing bodies which have already been postulated to act as lysosomes (Cronshaw 1964; see also O'Brien and Thimann 1967) it is reasonable to suggest that these *may* be the source of the hydrolases.

If this line of reasoning is correct, it is necessary to enquire into the mechanism which prevents lysis of the walls of the adjacent cells. The dissolution seems to stop at about the middle lamella, although this also disappears in the interband region if two vessel elements are contiguous (Fig. 12). The material in the intercellular substance also resists dissolution in many instances. Scott et al. (1960) claimed that the intercellular substance (ICS) of the xylem in *Ricinus* was suberized, and Sorokin (1958) has shown that similar material in peas contains a high content of alcohol-soluble material. Unfortunately, the ICS around these vessels in the coleoptile fails to stain at all with sudan black B, even in fresh material, so that one can probably not attribute the resistance to dissolution of this ICS to its lipid content. Perhaps the pectic substances, either directly or indirectly (e.g., by the presence of bound metals) inhibit the hydrolases. Lignification of the wall clearly inhibits dissolution, for the bands of thickening are quite strongly PAS-positive (see below). Furthermore, Ruesink's observations (1966) on the stages of protoplast formation demonstrate the resistance of the lignified bands to cellulase attack. It is much more difficult to visualize what prevents the lysis of the primary wall in the regions beneath the bands (Fig. 12–13); since this material is removed on some occasions, however, the limitation may be only one of diffusion.

The complete loss of the cytoplasmic contents of mature tracheary elements is an observation of long standing of a dramatic event upon which very little attention has been focused. Indeed, much more attention has been devoted to attempts to understand the pattern in which this death occurs than to the mechanism by which the cell-contents are "removed." If bac-

Figs. 32–34. The xylem parenchyma cells. The inset on Fig. 32 is a light micrograph, phase contrast, which shows that the aggregates of rough ER (such as that seen in Fig. 34) can be visualized in the light microscope. This is not an adjacent section, however. Note how the band of thickened wall has been deposited over the plasmodesmata (*PL*). The ER is richly invested with polyribosomes, especially evident when seen in surface view. Fig. 33 shows the unusual appearance of the wall/cytoplasm interface, commonly seen in cells which abut the tracheary elements. Note the hydrolyzed wall (HW). Fig. 32: ×29,400; inset: ×1,050. Fig. 33: ×28,600. Fig. 34: ×34,000.



Figs. 32–34.

teria are not responsible (and it does not seem likely) then autolysis must be the cause. It may well be that "hemicellulases" are *always* released during autolysis in these tracheary cells. The conclusion that such enzymes are released in at least some cases, e.g., in the formation of the perforation plate of vessels (Esau 1943) and in the cells of the coleoptile described here, would seem to be inescapable.

The observations presented here suggest that two types of material may protect the wall from hydrolysis, namely lignin, and an as yet undetermined component which must be present in the middle lamella and in ICS. To these observations, one must add one more fact, namely, that lignification does not seem to occur in a primary wall which is able to extend, either actively or passively; when a primary wall does become lignified, the lignification occurs after extension is completed (Bierhorst 1960). The reason seems obvious enough, for a lignified wall cannot be extended to any appreciable extent without rupture. Armed with these facts it is interesting to examine a few examples of the structure of tracheary elements.

When vessels are formed in the secondary xylem, the lateral primary walls are protected from attack by lignin, as are any bands of secondary material which have been laid down in the cross wall. Perforation occurs by loss of the non-cellulosic polysaccharides in the regions between the lignified layers of the wall. As is well known, the exact pattern of the perforation plate varies greatly (Esau 1965 a), and a true perforation, i.e., an area which also has lost the cellulose residue, is often confined to the larger spaces between areas of secondary thickening (Esau 1965 a, plate 36 C and D). It is of course possible that cellulose is removed by a cellulase in this situation, but it is also possible that the removal of this rather sparse mat of cellulose fibrils is accomplished mechanically.

The structure of the bordered-pit/torus complex of pines is also suggestive. Here, the secondary and primary walls are completely lignified except at the region of the pit membrane. The torus consists of the polyuronide-rich material which we have already postulated resists attack. The margins of the pit membrane, between the torus and the primary wall which underlie the bordered margins, are hydrolyzed to varying extents, leaving a more or less perforated, wagon-wheel arrangement of the cellulose fibrils (Bailey 1957). Apparently this is a special modification produced by the same basic system as that which creates perforation plates in vessels, and it is consistent with the suggestions outlined above.

A difficult question remaining is: what is the state of the lateral walls in *tracheids* of protoxylem? It is impossible to decide from the existing literature whether the lateral walls have lost their non-cellulosic polysaccharides in the inter-band regions of such cells or not, because the work has

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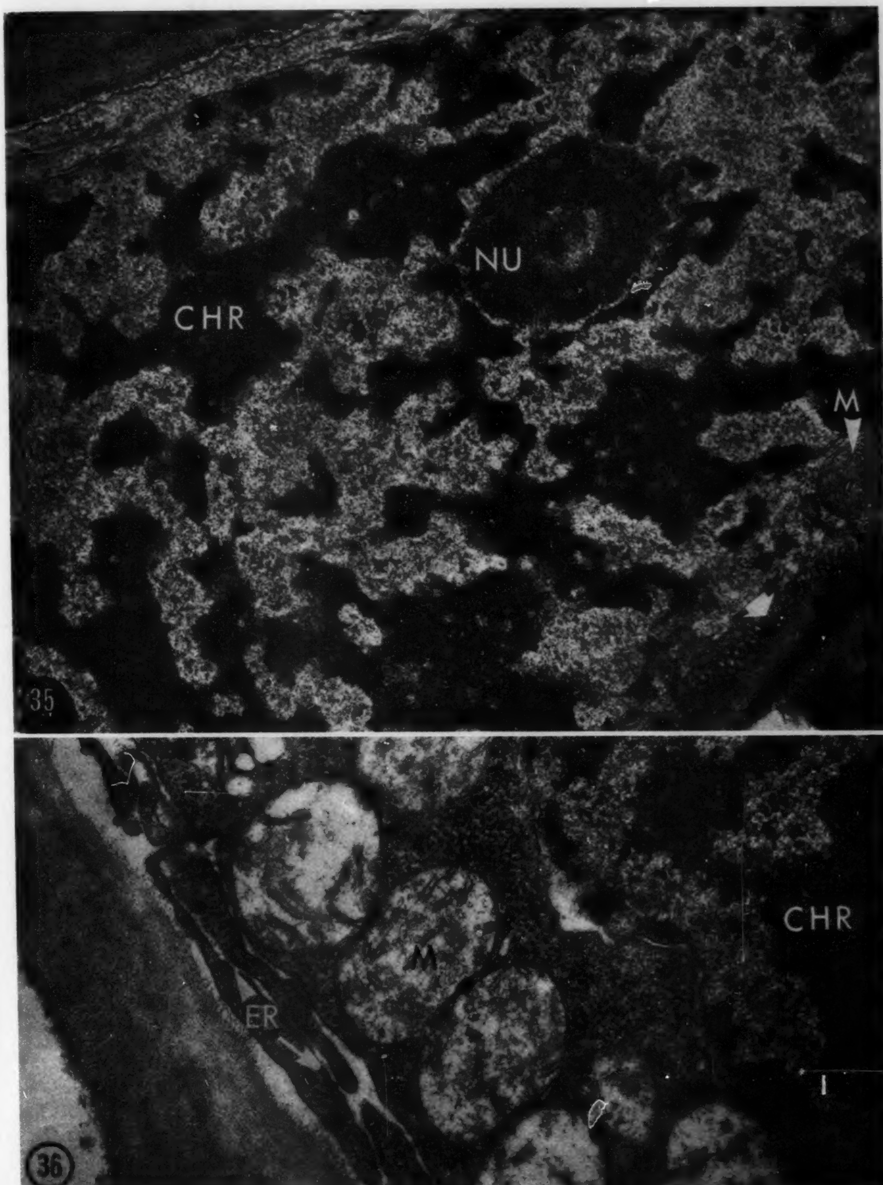
Figs. 35 and 36. Two companion cells, one younger (Fig. 35) than the other. Note the intact mitochondrion in Fig. 35, and the extraordinary chromaticity of the nucleus. In Fig. 36 the ER appears somewhat swollen, and the ground substance is somewhat increased in electron contrast. This corresponds with a loss of the mitochondrial stroma. Fig. 35: ×29,400. Fig. 36: ×42,000.



Figs. 35 and 36.

been based upon macerated material (Bierhorst 1960). If they are hydrolyzed as in the vessel elements reported here, the simple hypothesis erected above will explain it. If they are not hydrolyzed, the hypothesis might need to be amended to include the possibility that the composition of tracheids is fundamentally different from that of vessels. Perhaps the cells might lack the hemicellulases necessary to attack the wall, or perhaps the walls are protected by an enrichment of the material similar to intercellular substances, which protects tori and middle lamellae from digestion. As yet, no direct tests have been made of these suggestions. Nevertheless, if the facts reported here are confirmed in other systems (and we have already shown that the same structures occur in the vessels of rye coleoptiles), the consequences would be far-reaching. In addition, it is worth while pointing out that the fibrillar residue which is left in these elements is birefringent, contains no free carboxyl groups, and is PAS-negative. This residue may well be worth intensive study, since it represents the remains of a wall degraded by a natural population of enzymes. No attempt has yet been made to determine whether it contains protein (Lamport 1965) or the nature of the fibrillar material which is seen in the electron microscope.

Finally, it is necessary to comment upon the unusual staining behavior of the lignified bands of thickening in these coleoptile vessels. Although lignin may stain with Schiff's reagent, the intensity of the PAS-reaction of the lignified wall studied here is very greatly enhanced by periodate oxidation. Since many lignified walls fail to give the PAS-reaction (e.g., the stelar tissue of Smilax root), the PAS-positivity of this wall in the coleoptile vessels is probably due to accessibility of its non-cellulosic polysaccharides to periodate oxidation. Furthermore, although the majority of

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Fig. 37. The parenchyma cells which form the remnant of the bundle sheath. ×12,700.

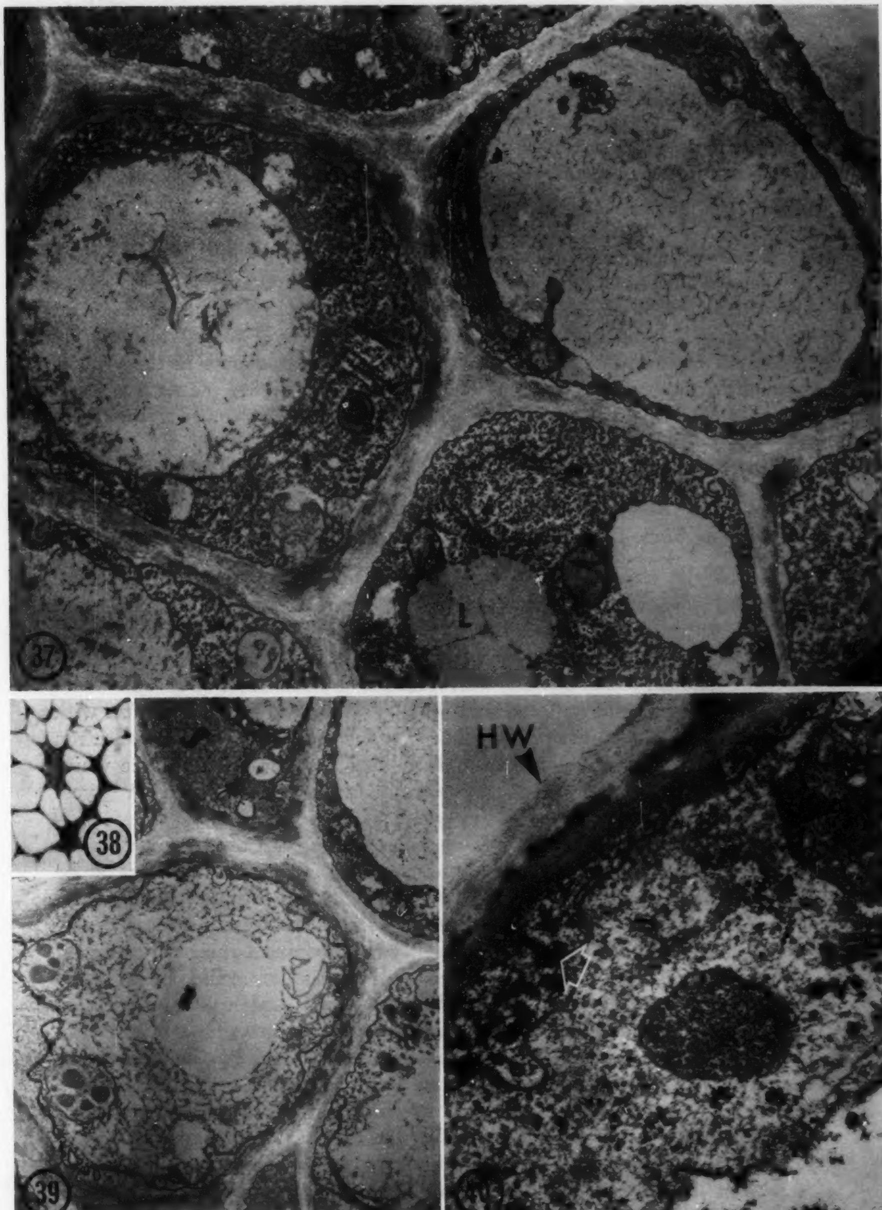
Fig. 38. Light micrograph of the obliterated phloem (PAS-stain). ×1,050.

Fig. 39. Degenerating phloem cells in the region near to that shown in Fig. 38. Note the swelling of the nacré walls, and the way this material seems to encroach upon the lumen of the cells. ×11,500.

Fig. 40. A hydrolyzed wall (*HW*), and unusual activity of the cell membrane in a xylem parenchyma cell of a rye coleoptile. Compare with Fig. 33. ×40,500.

Fixation procedures: Figs. 1–11; 26; 32, inset: Acrolein at 0° C. Figs. 12–14; 15 and inset; 16–18 and insets; 19 and inset; 20 and inset; 21–25; 27–30; 32; 33; 35–40: Glutaraldehyde/OsO_4. Fig. 34: Acrolein/OsO_4.

Abbreviations to legends: cc = companion cell; *CCB* = crystal containing body; *CHR* = chromatin; *CR* = crystalloid; *DSE* = differentiating sieve element; *ER* = endoplasmic reticulum; *HW* = hydrolyzed wall; *IE* = inner epidermis; *IS* = intercellular substance; *L* = lipid droplet; *LW* = lignified wall; *M* = mitochondrion; *ML* = middle lamella; *MT* = microtubule; *N* = nacré; *NU* = nucleolus; *OE* = outer epidermis; *P* = plasmodesmata; *PL* = plastid; *PR* = polyribosomes; *PW* = primary wall; *ST* = sieve tube; *STM* = sieve tube member; *STR* = sieve tube reticulum; *TE* = tracheary element; *XP* = xylem parenchyma cell.



Figs. 37–40.

lignified walls will not react with the chlor-zinc iodide reagent to give the cellulose reaction, these vessel walls will do so. These facts add even further evidence to the growing list of indications that the chemical nature of lignin, its structure *in situ* in the wall, and the manner in which it is intercalated with the other wall materials, vary considerably in different plants, and even in different organs and cell types (Freudenberg 1964; L.M. Srivastava, personal communication).

B. The Sieve Tubes

There seems to be little point in entering upon any detailed discussion of the structure of the mature sieve tube for it is abundantly clear from the literature (see Esau 1965 b; 1965 c; Srivastava and O'Brien 1966 for references) that no one has yet fixed these cells in a form which closely resembles their *in vivo* state. Since long-distance transport in the phloem undoubtedly plays a key role in the physiology of higher plants, and since the nature of this transport process is so surrounded by controversy, surely a successful solution to the structure of the mature sieve tube must be regarded as one of the most urgent problems in the cell biology of higher plants?

C. The Sieve Plate, Sieve Field and Differentiating Sieve Element

All of the observations presented here on the structure of the sieve plate and sieve field, and the development of the sieve plate, are consistent with the suggestion that these areas arise by modifications of the non-cellulosic wall polysaccharides around the plasmodesmata. The regions of low electron-contrast which initially surround plasmodesmata (Fig. 28) and which eventually seem to be replaced by a pore (Figs. 20 and 21) fail to stain with the PAS-procedure and with toluidine blue 0. This material of low electron contrast may not be "callose" because in thin sections of this tissue which have been fixed in acrolein or glutaraldehyde and embedded in glycol methacrylate, the sieve plates do not stain with aniline blue.

Since the non-cellulosic polysaccharides of the wall are removed in the region of the sieve pore, it seems that once again hemicellulases must be active in the cell. However, it is not easy to deduce whether they are released to a local region of the wall, or whether they are distributed generally throughout the cell but the wall is protected from their action in all regions except near plasmodesmata. This protection could be afforded either by the plasmalemma or by the nacré wall, and it is impossible to decide between these on the basis of present evidence. One must again postulate that the middle lamella constitutes a special barrier because in the sieve fields which connect STM to parenchyma cells, the parenchyma cell side of the pit shows no apparent difference from that of a normal primary pit-field. Obviously, something confines the activity of the enzyme to the sieve-element side.

One of the most consistent features of sieve element differentiation is to be found in the steadily increasing number of reports in which some major modification of plastid structure has been found (see Esau 1965 b, 1965 c;

and Srivastava and O'Brien 1966 for bibliographies). It is perhaps not surprising that the plastids which are contained in the osmotic environment of a sieve tube (probably not less than 0.5 M. sucrose solution) should be strongly modified: indeed, one would be hard pressed to explain how they could remain normal! When the fixation is very gentle, the majority of these highly abnormal plastids remain intact (Esau 1965 b) but in a good many cases, at least some of them are found ruptured (e.g., Falk 1964; Srivastava and O'Brien 1966). The well-known ability of mature sieve elements to heal a wound by a massive deposit of callose (Esau 1965 a) may have its explanation in these bodies. It was simple to confirm the fact that at sieve plates which were near a cut surface, the plate was covered with a mass of these plastids, in which many free granules were present. But these tissues were fixed in acrolein which penetrates the cells very rapidly and is extremely toxic to enzymes, and indeed, no callose could be detected on these areas. It does seem likely that the rupture of the plastids may release an enzyme which would form callose; its absence in acrolein-fixed tissues may merely afford confirmation of the evidence already presented by Evert and Derr (1964) that the amount of callose present on the plates is a function of how rapidly the tissue is killed by the fixative.

No firm conclusions about the nature of the sieve pores *in vivo* can safely be drawn from these or any other observations based on fixed material. Whether the pores are completely open, or completely occluded *in vivo* by cell content can obviously not be determined if fixation was accompanied by a major turgor release (as in most observations in the literature, including our own). Even when attempts have been made to prevent the turgor release (e.g., by fixing in strong sugar solutions), it is still possible that the cells have undergone the "surging motion" described by Currier et al. (1955), so that one should not place too much reliance upon the final state visualized.

D. The Companion Cells

In 1957 Esau et al. stated that "the subject of the parenchyma in the phloem is obviously in need of further exploration." The subsequent decade has added little of significance to our understanding of these cells. Many investigators have confirmed that the companion cells possess the necessary "biochemical machinery" (in particular, numerous mitochondria) to fulfill their postulated rôle as "accessory cells" to the sieve tubes, and the observations presented here add further support to this view. However, the exact biochemical activities of these cells remain unknown.

In the majority of cases studied here, the companion cells in the upper part of the bundle of a mature coleoptile are of the very acidophilic type whose mitochondria seem to have lost their stroma (see Figs. 31 and 36). One of the observations upon which the idea of a "physiological association" between companion cells (or other parenchyma cells) and sieve elements is based is the fact that they seem to die together. If one accepts the idea that the companion cell is essential to the proper functioning of

the sieve element with which it is associated (although some sieve elements appear to lack companion cells), this loss of mitochondrial stroma might be one of the primary factors responsible for the senescence of the unit.

E. The Xylem Parenchyma

Few cell-types have been so little explored: indeed, there is a strong tendency to regard them as what is "left over" when one has accounted for the more elaborately-patterned, but dead, tracheary elements. Yet there are reasons for thinking that these cells may turn out to be among the most important in the plant. They contain numerous mitochondria (indeed, per unit volume of cytoplasm they are about as rich in these organelles as the companion cells), which have very well-developed cristae and a large number of the osmiophilic granules. These features are obviously consistent with the suggestion that they are involved in salt accumulation (see also the discussion of this possibility for epidermal cells by O'Brien 1967). Their position next to the transpiration stream affords these cells a unique opportunity to accumulate material from it, and to modify its contents.

They also contain very dense aggregations of cisternal rough ER, which, in surface view, is densely covered with polyribosomes. This suggests activity in protein synthesis, and certainly, these cells are well-placed to accumulate the amino and amide nitrogen which is present in the transpiration stream. If so, they could play a major rôle in regulating the physiology of the organ by exercising control over the ionic and nitrogen content of the transpiration stream. In any event, they are certainly important as storage cells, especially in secondary tissues, and in various situations, they have been shown to contain all known forms of ergastic substance (Esau 1965 a). In the coleoptile, they are unusually rich in lipid.

The problem of the fate of the cytoplasmic contents of differentiating tracheary elements was raised above in connection with the strange appearance of the cortex/wall interface in some parenchyma cells. It was suggested that this might reflect micropinocytotic activity associated with the recovery of the hydrolyzed contents. There is, however, another possible explanation. The material outside the membrane in Fig. 40 closely resembles the middle lamella in electron contrast. We have already stated that at least some component of the middle lamella resists attack by the enzymes that remove the hemicelluloses from the vessel wall, and that this protection is not complete. It is possible that this activity represents a reaction of these parenchyma cells to the presence of such enzymes in the cell nearby, and that the response leads to the elaboration of more of the material which will protect the parenchyma cell wall from attack.

F. General Comments

Fine structure studies have their limitations. The amount of tissue that any one investigator may examine, even in a working lifetime, is infinitesimally small; the sampling problem is acute. Under ideal conditions of tis-

sue preparation, only the macromolecules are left in the specimen, and furthermore one knows that the architecture observed represents only that structure which obtained at the time of fixation. Even a brief look at the dynamic state of the living cell should convince the most optimistic that the relationship between the static image and the dynamic reality is rather uncertain. These problems are, of course, basic to all histology but are severely enhanced in EM by the technical limitations which surround electron optics (thin specimens of small area, high vacuum, etc.).

Faced with such difficulties, it might seem naïve to hope that much progress can be made toward elucidating the all-important relationship between structure and function. Yet, in the better-studied systems, especially in bacteria and in vertebrate tissues, there has been real progress. In the botanical field, however, outside of the studies of the chloroplast, progress is painfully slow. Several factors contribute to this state of affairs.

First, plant tissue is more refractory than most vertebrate tissue. The thick cell-wall, the hydrostatic pressure of the vacuole, and the relatively low protein-content of the cytoplasm in many cell-types pose problems in all stages of specimen preparation.

Second, and even more serious, is the conservative attitude of many investigators toward new techniques of specimen preparation. The more refractory nature of the material actually demands a *more critical* attitude toward techniques than would be necessary if the material were simple to study. In this connection, material fixed in permanganate deserves special comment. The image produced in plant material by this "fixative" is well known. It is beautifully diagrammatic, for the only material which seems to be well-preserved are the membranes of the various organelles. Almost no detail can be seen in the cell-wall (unless it is lignified), nucleus, or nucleolus; the stroma material of plastids and mitochondria, the ribosomes, and the bulk of the ground substance are not preserved. If it is difficult to attempt to relate structure to function when *all* of the major macromolecular species are preserved within the cell, surely it is much more so when the bulk of these materials has been extracted or rendered unrecognizable? Somewhat similar considerations apply to most plant tissues which have been fixed solely in OsO_4. "Densely-cytoplasmic" cells, such as companion cells or root tip cells, are reasonably well preserved, but vacuolated parenchyma cells are very poorly preserved relative to the image of the same tissue prefixed in an aldehyde (see, e.g., Ledbetter and Porter 1963; Cronshaw and Bouck 1965; Gunning 1965 a).

Naturally much work was carried out prior to the introduction of aldehyde/OsO_4 fixation by Sabatini et al. (1963), but nevertheless the present studies make clear that there is little point in continuing to attempt fine structural analysis of a tissue (even one as well explored as the coleoptile) by any methods which fail to preserve the bulk of the cytoplasm.

Finally, too little attention has been given to the correlation of light and electron microscopy, especially upon tissues fixed in the same or similar ways. The optical beauty of sections 0.25–1.0 μ thick for general histology was pointed out six years ago by Richardson et al. (1960). Such sections

reveal an unexpected wealth of detail and, with a little practice, one soon learns that many structures which are normally classed as sub-microscopic (e.g., the ER), can readily be visualized.

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Protoplasmatologia. Handbuch der Protoplasmaforschung. Begründet von L. V. Heilbrunn und F. Weber. Band V: Karyoplasma (Nucleus). 3. Chemistry and Cytochemistry of Nucleic Acids and Nuclear Proteins. a—d. By C. Scholtissek, B. M. Richards, R. Vendrely, C. Vendrely and D. P. Bloch. With 67 Figs., IV, 236 p. Wien-New York: Springer-Verlag. 1966. S 510.—. Vorzugspreis bei Verpflichtung zur Abnahme des gesamten „Handbuchs der Protoplasmaforschung" S 410.—.

Der vorliegende Band enthält vier Beiträge zur „Chemie und Biochemie von Nucleinsäuren und Kernproteinen".

1. „The Chemistry and Biological Role of Nucleic Acids" (Ch. Scholtissek, Tübingen). Nach einem kurzen Abriß der geschichtlichen Entwicklung der Nucleinsäurechemie folgen vier Kapitel über Chemie, physikalische Eigenschaften, Biochemie und Stoffwechsel und Funktion der Nucleinsäuren. Zu einem prägnanten Text bilden übersichtliche Formelschemata und Tabellen eine gelungene Erweiterung und Vertiefung.

2. „Cytochemistry of the Nucleic Acids" (B. M. Richards, London). Dieser Beitrag enthält die allgemeinen Grundlagen und Methoden der Mikrospektrophotometrie im UV- und sichtbaren Bereich sowie der licht- und elektronenoptischen Autoradiographie. Ausführlich werden dabei die Fehlerquellen der einzelnen Methoden und die Möglichkeiten der Fehlinterpretation von Versuchsergebnissen dargestellt. Für die quantitative Cytochemie der Nucleinsäuren gewinnen diese Verfahren wachsende Bedeutung; die hierbei bestehenden Probleme werden in einem gesonderten Abschnitt betrachtet.

3. „Biochemistry of Histones and Protamines" (R. Vendrely und C. Vendrely, Villejuif). Die Verfasser beschreiben Methoden zur Darstellung von Histonen und Protaminen und zur Fraktionierung von Histonen, die Zusammensetzung dieser Kernproteine und einzelner Fraktionen, die wahrscheinliche Struktur, Gewebe- und Spezies-Spezifität sowie die Funktion und den Stoffwechsel der Histone.

4. „Cytochemistry of the Histones" (D. P. Bloch, Austin). Neben der Darstellung der cytochemischen Methoden zum Nachweis von Kernproteinen enthält dieses Kapitel Beiträge zum Stoffwechsel der Histone sowie ihre Beziehungen zur DNS und zur Chromosomenreplikation und -differenzierung. Zusammen mit den vorstehenden Ausführungen über die Biochemie dieser Verbindungen wird so ein guter Überblick über die Kernproteine unter den verschiedensten Gesichtspunkten gegeben.

Bei der stürmischen Entwicklung dieser Gebiete in den letzten Jahren ist es verständlich, daß die Arbeiten nur einzelne Aspekte behandeln; viele Fragen können nur angedeutet, andere gar nicht erwähnt werden. Ein umfangreiches Literaturverzeichnis am Ende eines jeden Artikels ermöglicht aber dem Interessierten eine umfassende Information. Auf diese Weise wird das Werk sowohl dem Spezialisten als auch dem allgemein interessierten Leser eine große Hilfe sein.

D. Neumann (Halle)

Crew, F. A. E.: The Foundations of Genetics. With 23 figs., XIII, 202 p. Oxford-London-Edinburgh-New York-Toronto-Paris-Frankfurt: Pergamon Press Ltd., 1966. 21 s.

Dieses Taschenbuch bietet in lehrreicher Form eine Einführung in die moderne Genetik vom Aspekt ihrer geschichtlichen Entwicklung. Nach einer Übersicht über die Züchtungsforschung vor Mendel wird das Leben und das Werk Gregor Mendels eingehend gewürdigt. Über die Wiederentdeckung der Mendelschen Regeln und die Entfaltung der kreuzungsanalytischen Forschung wird der Leser zum Verständnis des klassischen Genbegriffs geführt und damit zur Grundlage der modernen, vielseitigen Entwicklung der Genetik. Ihrer Untergliederung in methodisch differenzierte Teilgebiete und deren Synthese sind die letzten Kapitel dieses sehr instruktiven und leicht lesbaren Buches gewidmet.

F. Mainx (Wien).

Bacci, G.: Sex Determination. With figs., VIII, 306 p. Oxford-London-Edinburgh-New York-Toronto-Paris-Frankfurt: Pergamon Press Ltd. 1965. 84 s.

In wohldurchdachter Weise wird die große Mannigfaltigkeit von Erscheinungsformen der Sexualität und der Geschlechtsbestimmung in allen Gruppen des Organismenreiches übersichtlich dargestellt. Etwa 800 Literaturzitate beweisen die Fülle der auf diesem Gebiet geleisteten Arbeit. Nach einer Einleitung über die Fortpflanzungszyklen verschiedener Organismen und einem Kapitel über die protosexuellen Prozesse bei Bakterien und die Sexualität bei niederen Organismen werden die genetischen und cytologischen Grundlagen der genotypischen Geschlechtsbestimmung besprochen, ferner die Erscheinungen der Intersexualität und die Balance-Theorie des Geschlechts. Weitere Kapitel behandeln die komplizierten Mechanismen von polygener und progamer Geschlechtsbestimmung, die Fragen des Hermaphroditismus, seiner Balance und seiner Zusammenhänge mit Umweltfaktoren, ferner die Fälle von Geschlechtsumwandlung und den Spezialfall der männlichen Haploidie. Geschlecht und Heterogonie, Parthogenese und ähnliche Erscheinungen werden ausführlich behandelt. Die Bedeutung der Sexualität für die Evolution und die Entstehung der verschiedenen Geschlechtsbestimmungs- und Geschlechtsdifferenzierungstypen werden erörtert. Es ist einer der Vorteile dieses Buches, daß Beispiele aus dem Tier- und Pflanzenreich in gleicher Weise berücksichtigt werden. Der Autor, dem wir eine Reihe von wertvollsten Experimentalarbeiten auf dem Gebiet verdanken, vermeidet jede überflüssige Spekulation, würdigt aber mit Recht die Bedeutung der Weismannschen Amphimixistheorie in ihrer durch die moderne Genetik und Evolutionsforschung bereicherten Form.

F. Mainx (Wien).

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PROTOPLASMA

IN VERBINDUNG MIT R. CHAMBERS UND W. SEIFRIZ

BEGRÜNDET VON FRIEDL WEBER UND JOSEF SPEK

FORTGEFÜHRT VON KARL HÖFLER

UNTER BESONDERER MITWIRKUNG VON

R. BIEBL, WIEN · J. BRACHET, BRUXELLES · N. KAMIYA, OSAKA · H. KINZEL, WIEN

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Die Entstehung der Arten und höheren Kategorien

Experimenteller Nachweis des Ablaufs der Evolution

Von Prof. DDr. et Dr. h. c. Herbert Lamprecht
Graz und Landskrona

Mit 110 Textabbildungen. XI, 452 Seiten. Gr.-8°. 1966
Ganzleinen S 490.—, DM 78.—, $ 19.50

Sind Arten, Gattungen usw. naturbedingte Realitäten, und welche sind die hierfür verantwortlichen Grundlagen? Wie kann der Schritt von einer zur nächsten Spezies, Gattung usw. im Verlauf der Phylogenese geschehen sein?

Gestützt auf die Ergebnisse von über 30 Jahren experimenteller Forschungsarbeit, legt der Verfasser die eindeutige Beantwortung dieser Fragen vor. Das Alpha und Omega ist hierbei die Grundlage der unüberbrückbaren Barriere zwischen den systematischen Kategorien, die in ihrer Abhängigkeit von genetischen, zytologischen und plasmatischen Faktoren geklärt wird. Erwähnt sei hier nur die Wirkung interspezifischer Gene, deren Allele stets auf verschiedene Kategorien verteilt sind, sowie das Vorhandensein eines artspezifischen Plasmas für deren Synthese. Die Ergebnisse des Verfassers geben auch den experimentellen Weg zur Feststellung der Morphologie ausgestorbener und fossil unbekannter Organismen an. Die Entstehung des Menschen aus Pongiden findet eine einfache und natürliche Erklärung.